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# Advances in temperate grassland science and management

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# Advances in *Epichloë* endophyte plant secondary metabolites in grasslands

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## 1 Introduction

Two of the most widely cultivated cool-season forage grasses in mesic, temperate, perennial grasslands are tall fescue (*Festuca arundinacea* syn. *Lolium arundinaceum*) and perennial ryegrass (*Lolium perenne*) (Easton *et al.*, 1994; Jung *et al.*, 1996; Fribourg *et al.*, 2009; Ball *et al.*, 2019). These grasses

are adapted to a range of climatic conditions and various soils, tolerate a wide range of grazing managements and cutting regimes, combine well with legumes and herbs in sown mixed-species pastures and in general provide nutritious feed for livestock such as dairy cows, beef cattle, sheep and horses. They became of particular importance in large areas of the *New World*, including countries of North and South America, and Australia and New Zealand, as European settlers began to farm livestock globally. However, in these regions over time, these grasses became associated with poor animal performance and health, in some seasons of the year (Hume *et al.*, 2016; Ball *et al.*, 2019). These detrimental effects on farmed animals were seldom apparent in the regions of Europe, North Africa and Asia where these grasses originated.

In the late 1970s and early 1980s, breakthrough discoveries in the USA and New Zealand found that the toxicity to livestock was due to asexual species of the *Epichloë* fungi that endophytically colonised tall fescue and perennial ryegrass (Bacon *et al.*, 1977; Fletcher and Harvey, 1981). The naturalised, well-adapted local grass populations were highly infected with asymptomatic mammalian toxic strains of *Epichloë*, having been unknowingly introduced from the centres of origin by the European farmers (Johnson *et al.*, 2013). Of equal importance was the discovery that *Epichloë* were also providing protective properties to their grass hosts, which in some cases were the key determinant of their persistence in these countries (Mortimer *et al.*, 1982; Read and Camp, 1986). For tall fescue, this manifested as a greater tolerance to drought and pests such as nematodes, and for ryegrass this was due primarily to less damage caused by several invertebrate pests (Hume *et al.*, 2016; Ball *et al.*, 2019; Caradus *et al.*, 2021b; Caradus, 2023a).

The anti-quality effects for livestock, and many of the bioprotective properties of the grass, can be attributed to bioactive secondary metabolites produced by the endophyte when in association with the grass host. The bioactive metabolites are produced by both the asexual and sexual species of *Epichloë*, which collectively infect 29 genera of temperate grasses spread across all six farmed continents of the world (Schardl *et al.*, 2013a; Schardl *et al.*, 2013b; Leuchtman *et al.*, 2014; Caradus *et al.*, 2021a; Anon., 2023b).

In this chapter we describe the biology of the *Epichloë*-grass association, the secondary metabolites that are linked to the bioactivity, control of invertebrate pests, the occurrence of mammalian toxicity globally, toxic responses in terms of animal productivity, health and welfare, utilisation of *Epichloë* strain variation in commerce, along with flow-on effects to animal product quality and food safety. There is a particular focus on the most well-studied, mutualistic tall fescue and perennial ryegrass endophyte associations due to their significant economic impacts and prospects to advance future sustainable grassland farming.

## 2 *Epichloë* biology

*Epichloë* is a filamentous fungal genus within the tribe Balansieae (family Clavicipitaceae). Over many millennia, *Epichloë* have co-evolved with their temperate (cool season) grass hosts within the sub-family Pooideae (Schardl *et al.*, 2008; Schardl, 2010). Over this period of co-evolution, *Epichloë* have developed a high degree of host specificity which in nature generally restricts each *Epichloë* species to a single or closely related grass genera, although there are notable exceptions. *Epichloë* have been documented in over 100 grass species from a number of the tribes within the Pooideae sub-family (Caradus *et al.*, 2021a).

As an obligate symbiont, *Epichloë* can only live within a grass plant and seed from that plant. In vegetative plants, the growth of *Epichloë* is fully synchronised and highly regulated by the host grass (Christensen *et al.*, 2002; Christensen and Voisey, 2007b; Christensen *et al.*, 2008; Voisey, 2010). *Epichloë* hyphal strands colonise the apical meristem of the vegetative grass, through extensive hyphal branching between the grass cells. This enables the fungus to colonise the meristems of new tillers and inflorescence primordia as they develop. Fungal tip growth occurs in meristematic tissue, while a unique process of intercalary hyphal extension occurs in the leaf. Intercalary growth allows hyphae to grow with the leaf when it is in the phase of rapid elongation. As the leaf matures, hyphae branch through tip growth, where it occupies the intercellular spaces of the leaf sheath and to some extent leaf blade. In most cases, hyphal mass decreases with increasing distance from the crown of the plant. While *Epichloë* are systemic in the shoot, there have only been rare observations in roots, and they are not present in pollen (Siegel *et al.*, 1984; Schardl *et al.*, 2004; Christensen and Voisey, 2007a).

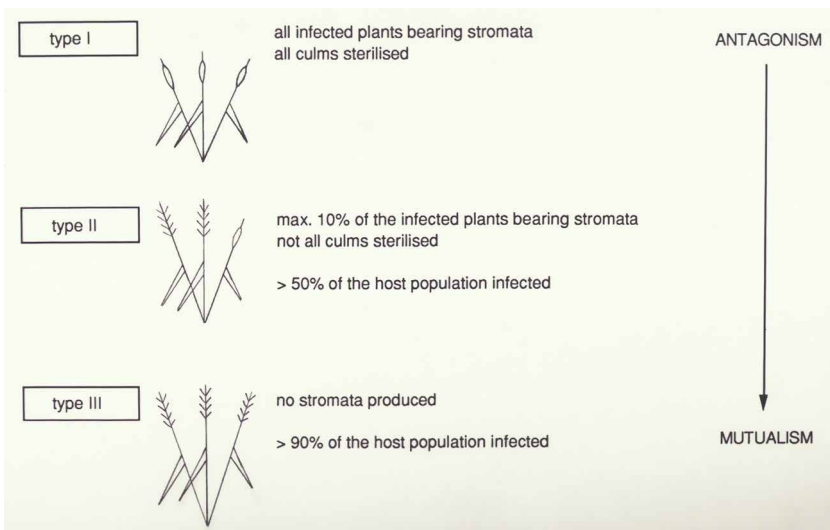
When a grass host becomes reproductive, hyphal growth varies between asexual and sexual species of *Epichloë*, affecting different paths of transmission for the fungus (Tadych *et al.*, 2014; Johnson *et al.*, 2021). Asexual species colonise the developing seed, particularly the seed embryo which is critical for the endophyte to continue its mutualistic, asymptomatic symbiosis when the seed germinates (Card *et al.*, 2011; Liu *et al.*, 2017; Zhang *et al.*, 2017). Some sexual species of *Epichloë* also colonise the seed and form new associations with the germinating seed. Transmission of *Epichloë* via the host seed is termed vertical transmission.

On the culms of reproductive tillers, sexual *Epichloë* may also form epiphytic reproductive structures (stroma) termed 'choke', as part of the process of sexual recombination and spread of *Epichloë* by ascospores. This is termed horizontal transmission. Grass associations with horizontal transmission are pathogenic for the host, as 'choke' prevents mature seed formation therefore preventing sexual propagation of the grass. Asexual *Epichloë* grass associations are always

asymptomatic, while the presence of choke is a clear symptom of a sexual *Epichloë*-grass association.

On the basis of the form of transmission and reproduction, *Epichloë*-grass associations can be classified into three types (White, 1988; Leuchtman and Clay, 1997) (Fig. 1). For type I associations, 'choke' occurs on all seed heads which completely prevents the grass from producing seed. In these associations, there are restrictions on reproduction for both symbionts which are likely to contribute, along with other factors, to type I grass-endophyte populations remaining small. For type II associations, not all individuals in a grass population, and not all reproductive tillers on a plant, are affected by choke. In this case, the endophyte can propagate not only via sexual reproduction but also vegetative reproduction, as it infects the grass seeds on tillers without choke. In type III associations, the asexual endophytes do not form choke in the seedheads. This allows for the full expression of the host's sexual reproductive capacity. However, the endophyte is clonal and needs to adapt to the new genetics of the grass progeny, which may not be so favourable for the endophyte.

In an agricultural context, the negative impact of sexual endophytes on seed yields is a disadvantage (particularly type I associations) of what is otherwise a mutualistic symbiosis. While each type of grass-endophyte association has varying pros and cons during this reproductive phase, for both organisms, of the 48 *Epichloë* described as of 2022, the majority are asexual (33 type III



**Figure 1** Types of endophyte-grass associations formed by sexual (types I and II) and asexual (type III) forms of *Epichloë*. A continuum of interactions from antagonism (pathogenic) to mutualism occurring in the reproductive grass tillers. (Prepared by Erika Bucheli; provided courtesy of Adrian Leuchtman, ETH, Zurich, Switzerland).

associations)(Anon, 2023b). Of the 15 sexual *Epichloë*, just 4 could be classified as type I associations, the remainder being type II associations. The asexual associations have been of most interest to agriculture, possibly due to their high expression of mammalian and invertebrate toxic secondary metabolites (Leuchtman *et al.*, 2000). This high secondary metabolite expression is consistent with the defence mutualism theory (Clay, 1988; Saikkonen *et al.*, 2016). The asexual *Epichloë* needs to provide the host with as many protective properties as possible (e.g. via secondary metabolite production), as the endophyte is totally reliant on the survival of the host and spread via the host seed. Sexual endophytes can achieve spread via horizontal transmission, and with less secondary metabolite production this lowers any metabolic cost for the host.

### 3 Benefits for the host grass

Across the various *Epichloë*-grass associations, endophyte has been shown to improve the fitness of the plant under a range of abiotic and biotic stresses. As an obligate symbiont, *Epichloë* must improve host performance in some manner to ensure that infected plants are as or are more competitive than endophyte-free equivalents, and/or those of competing plant species, thus overcoming any metabolic cost for hosting the endophyte (Rodriguez *et al.*, 2009). In some cases, endophyte-infected plants may be inferior for some traits, which may be more so in natural than cultivated grass species (Faeth and Saari, 2012).

There are multiple ways by which the endophyte-driven increase in host fitness is achieved. For abiotic stresses, *Epichloë* has been shown to improve tolerance to drought, flooding, heavy metals, aluminium, nutrient deficiency, salinity, heat stress and winter cold (Malinowski and Belesky, 2000; Song *et al.*, 2015; Caradus and Johnson, 2020; Hewitt *et al.*, 2021; Caradus and Hume, 2023). For biotic stresses, plant pathogens may be reduced and of greatest importance is a reduction in herbivory by invertebrate pests and farmed animals (Xia *et al.*, 2018; Caradus and Johnson, 2020; Pérez *et al.*, 2020; Caradus *et al.*, 2021a; Card *et al.*, 2021). Various mechanisms are involved which include changes to plant morphology, physiology, levels of primary metabolites, interactions with other microorganisms and, in particular, bioactive secondary metabolites that are not produced in endophyte-free plants (Malinowski and Belesky, 2000; Caradus *et al.*, 2021a; Johnson *et al.*, 2021; Bastías *et al.*, 2024).

In an agricultural context, reduced herbivory is favourable in terms of invertebrate pests but not for grazing livestock as it manifests in poor livestock performance and serious health disorders. In addition, results from indoor experiments, or highly controlled field studies, may differ in the farm environment where multiple stresses may be active and multi-trophic

interactions occur (Fuchs *et al.*, 2020; Hewitt *et al.*, 2021). It may therefore be difficult to predict the endophyte-driven outcomes, particularly under a rapidly changing global environment (Bastías *et al.*, 2023).

The benefits of endophyte infection for the host grass reported in one grass species cannot be assumed to occur in another as each endophyte–grass association has unique characteristics. Even within endophyte species different strains may vary significantly in their secondary metabolite profile due to mutations. The discovery and development of endophyte strains lacking certain secondary metabolites have been utilised in commerce to achieve optimal agronomic performance with minimal or no negative effects on livestock (Caradus *et al.*, 2021a).

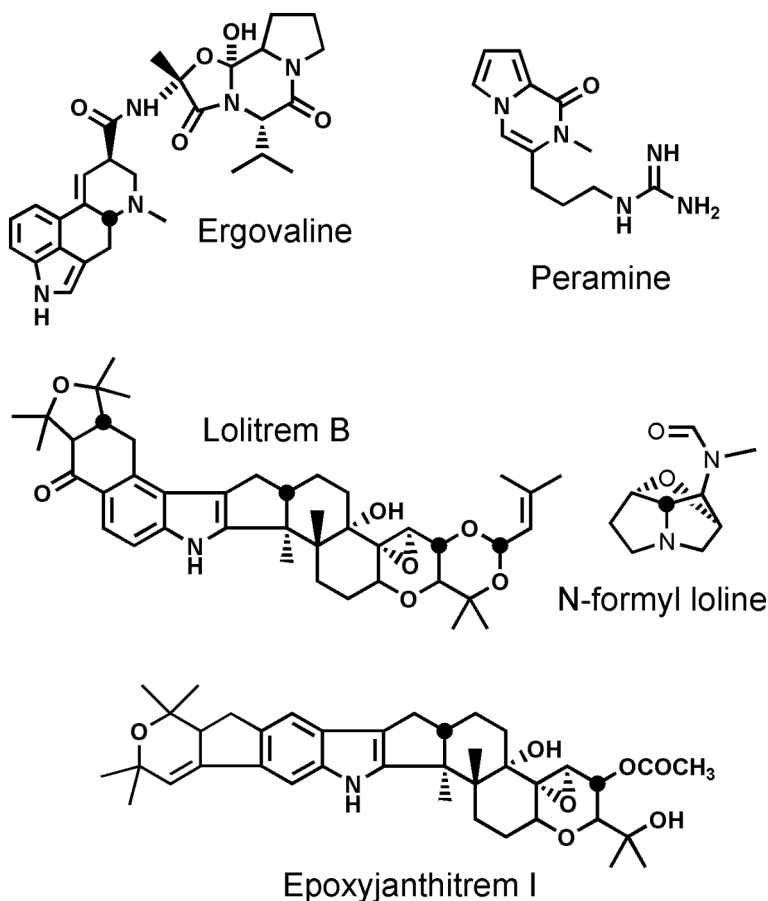
## **4 Secondary metabolites produced by endophyte–grass associations**

Endophyte–grass associations produce a myriad of secondary metabolites, with those associated with known bioactivity falling into four classes. These include indole-diterpenes (lolitrem B and epoxyjanthitrems), ergot alkaloids (ergovaline), pyrrolopyrazine alkaloids (peramine) and saturated 1-aminopyrrolizidine alkaloids (lolines) (Fig. 2). Endophyte-infected tall fescue is associated with ergot alkaloids (ergovaline is the major compound) (Lyons *et al.*, 1986), peramine (Rowan and Gaynor, 1986; Rowan, 1993) and loline alkaloids (N-formyl loline is the major compound) (Bush *et al.*, 1982). Naturally occurring perennial ryegrass is associated with lolitrems (lolitrem B is the major compound) (Miles *et al.*, 1994), ergot alkaloids (ergovaline is the major compound) (Caradus *et al.*, 2020) and peramine. However, to maximise the benefits of endophytes in commerce, novel selected endophyte–grass associations have been created for both tall fescue and perennial ryegrass (see section 10.4). This has led to the expression of further indole-diterpenes in perennial ryegrass, the epoxyjanthitrems (five compounds, epoxyjanthitrem I is dominant) (Fig. 2) (Finch *et al.*, 2020).

Rather than a single pathway end product, most of the secondary metabolite biosynthetic pathways also express intermediate compounds (Johnson *et al.*, 2021; Bastías *et al.*, 2023). While this appears to be inefficient, some of these intermediates have bioactivity that contributes to the diversity of defensive secondary metabolites in the plant (Panaccione, 2005; Schardl *et al.*, 2013b; Caradus and Johnson, 2020).

### **4.1 Factors affecting concentrations**

Concentrations of secondary metabolites in plant material are influenced by multiple factors including plant host, plant parts, season and other



**Figure 2** Major secondary metabolites produced by perennial ryegrass and tall fescue infected by *Epichloë* endophytes.

environmental factors (Agee and Hill, 1994; Easton *et al.*, 2002; Brosi *et al.*, 2011; Hennessy *et al.*, 2016; Fuchs *et al.*, 2017; Caradus *et al.*, 2020). In endophyte-infected ryegrass both lolitrem B and ergovaline are highest in mature seeds. In vegetative tissue, the endophyte is highest in the basal material corresponding to high lolitrem B and ergovaline concentrations. However, unlike ergovaline, lolitrem B is also high in old leaves and dead material (Repussard *et al.*, 2014). In contrast, peramine is more evenly distributed throughout the plant (Ball *et al.*, 1991). Although at a much higher concentration, ergovaline in endophyte-infected tall fescue shows the same distribution as that observed in ryegrass (Caradus *et al.*, 2020). Lolines are highest in seed heads and pseudostems compared to leaf blades (Justus *et al.*, 1997) and lolines are also found in root tissue (Patchett *et al.*, 2008b).

During winter, lolitrem B and ergovaline are at their lowest concentrations in endophyte-infected ryegrass, with both peaking in summer/autumn, but peramine concentrations remain relatively constant throughout the year (Ball *et al.*, 1991). In endophyte-infected tall fescue, both ergovaline and lolines are low in winter although concentrations peak at different times with ergovaline concentrations highest in late spring and lolines in summer (Bush *et al.*, 1993; Agee and Hill, 1994).

Temperature is a major factor influencing secondary metabolite expression with Hennessy *et al.* (2016) showing that endophyte-infected ryegrass grown at 20°C had much higher epoxyjanthitrem concentrations than plants grown at 7°C. Lolines in endophyte-infected tall fescue have also been shown to be affected by temperature (Huizing *et al.*, 1991) although this trend is less clear for ergovaline (Brosi *et al.*, 2011). Ryegrass grown at ambient CO<sub>2</sub> with added nitrogen showed an increase in peramine and ergovaline whereas the combination of elevated CO<sub>2</sub> and added nitrogen induced no effect on secondary metabolite production (Hunt *et al.*, 2005). In contrast, for tall fescue grown at elevated CO<sub>2</sub>, lolines and ergovaline decreased but added nitrogen increased ergovaline (Arechavaleta *et al.*, 1992). Although ergovaline in tall fescue is increased by high nitrogen, this effect was not seen with lolitrem B, peramine or ergovaline in endophyte-infected perennial ryegrass (Rasmussen *et al.*, 2007). Plant damage is another factor to consider since endophyte secondary metabolites are increased with plant wounding (Salminen and Grewal, 2002; Bultman *et al.*, 2004).

Additionally, water deficit has been shown to influence secondary metabolite expression, with lolines increasing significantly when tall fescue plants were under severe water deficit (Kennedy and Bush, 1983) although the evidence of an effect on ergovaline concentrations is conflicting (Belesky *et al.*, 1989; Arechavaleta *et al.*, 1992). The effect of water deficit on the secondary metabolites produced by perennial ryegrass is also uncertain with studies published in the literature reporting opposite effects. In one study, water stress was shown to increase the concentrations of lolitrem B and ergovaline but with no effect on peramine (Eerens *et al.*, 1998) whereas in another study drought stress was shown to increase peramine but not lolitrem B (Lin *et al.*, 2022).

## 5 Toxicity to invertebrate pests

Endophytes in grass associations provide a critical role in the protection of pasture from invertebrate pests (mainly insects) due to their production of secondary metabolites. This effect is vast, with 45 insect species from 10 different families affected (Malinowski and Belesky, 2019). The most important known secondary metabolites for pest protection are peramine and epoxyjanthitrem

in ryegrass and lolines in tall fescue. These compounds are 'beneficial' to the farming system because loline and peramine have no adverse effects on animals and the insect bioactivity of epoxyjanthitrems outweighs the negative ones on animals (see Section 6). In contrast, lolitrem B and ergot alkaloids are 'detrimental' to the farming system since they have severe effects on animals which outweigh their effects on insects.

The range of insects affected by the classes of secondary metabolites, as discussed in Section 4, are detailed in Table 1. The bioactivity listed in this table includes only that known to be induced by a specific metabolite, whereas the list of effects induced by endophyte-grass associations is far more extensive (Caradus and Johnson, 2020). For example, the AR37 strain of *Epichloë* that infects perennial ryegrass shows excellent bioactivity against a variety of insect pests including Argentine stem weevil (*Listronotus bonariensis*) (Popay and Wyatt, 1995), African black beetle (*Heteronychus arator*) (Popay and Thom, 2009), pasture mealybug (*Balanococcus poae*) (Pennell *et al.*, 2005), porina larvae (*Wiseana cervinata*) (Jensen and Popay, 2004) and a root aphid (*Aploneura lentisci*) (Popay and Cox, 2016). Epoxyjanthitrems are the only known secondary metabolites produced by this association and to date the correlation between this compound and insect bioactivity has been confirmed for only pasture mealybug and porina. The bioactivity of the AR37 endophyte against the other three key insect pests may be due to epoxyjanthitrems or it may be due to an unknown secondary metabolite.

## 6 Toxicity to livestock for major cultivated grasses

For cultivated grasses, the toxicity of endophytes to livestock are of greatest importance for tall fescue and perennial ryegrass where many, but not all, of the commonly occurring *Epichloë* strains of endophyte in these grasses produce indole diterpenes and/or ergot alkaloids.

### 6.1 Tall fescue toxicity

The toxicity of endophyte (*Epichloë coenophiala*)-infected tall fescue to livestock has been studied most in the USA due to the magnitude of the toxicity and its widespread occurrence, leading to significant economic impact. Ergot alkaloids, primarily ergovaline, cause three toxicity syndromes: fescue foot, bovine fat necrosis and fescue toxicosis, with fescue toxicosis having the greatest economic impact (Schmidt and Osborn, 1993; Strickland *et al.*, 2009; Waller, 2009).

**Table 1** The bioactive effects of secondary metabolites against invertebrate pests

Metabolite and insect	Effect	References
<b>Lolitrems</b>		
<i>Listronotis bonariensis</i> – Argentine stem weevil larvae	Deterrent and toxic	Dymock <i>et al.</i> , 1989
<b>Epoxyanthitrem</b>		
<i>Balanococcus poae</i> – Pasture mealybug	Reduced population	Pennell <i>et al.</i> , 2005
<i>Wiseana cervinata</i> – Porina	Antifeedant	Finch <i>et al.</i> , 2020
<b>Ergot alkaloids</b>		
<i>Agrostis ipsilon</i> – Black cutworm	Deterrent and toxic	Potter <i>et al.</i> , 2008; Baldauf <i>et al.</i> , 2011
<i>Heteronychus arator</i> – African black beetle adults	Antifeedant	Ball <i>et al.</i> , 1997b
<i>Spodoptero frugiperda</i> – Fall armyworm	Deterrent	Clay and Cheplick, 1989
<i>Pratylenchus scribneri</i> – Lesion nematode	Toxic	Bacetty <i>et al.</i> , 2009
<i>Listronotis bonariensis</i> – Argentine stem weevil larvae	Deterrent	Popay and Wyatt, 1995
<i>Popilla japonica</i> – Japanese beetle larvae	Deterrent	Patterson <i>et al.</i> , 1991
<i>Oncopeltus fasciatus</i> – Large milkweed bug	Toxic	Yates <i>et al.</i> , 1989
<b>Peramine</b>		
<i>Listronotis bonariensis</i> – Argentine stem weevil larvae and adults	Deterrent and toxic	Dymock <i>et al.</i> , 1989; Rowan <i>et al.</i> , 1990; Popay <i>et al.</i> , 2003
<i>Schizaphis graminis</i> – Aphid	Deterrent and toxic	Siegel <i>et al.</i> , 1990
<i>Balanococcus poae</i> – Pasture mealybug	Reduced population	Pennell <i>et al.</i> , 2005
<i>Agrostis ipsilon</i> – Black cutworm	Deterrent and toxic	Baldauf <i>et al.</i> , 2011
<b>Lolines</b>		
<i>Costelytra zealandica</i> – Grass grub	Deterrent	Patchett <i>et al.</i> , 2011a
<i>Heteronychus arator</i> – African black beetle adults and larvae	Deterrent	Barker <i>et al.</i> , 2015a
<i>Listronotis bonariensis</i> – Argentine stem weevil	Deterrent and toxic	Patchett <i>et al.</i> , 2008a; Popay <i>et al.</i> , 2009
<i>Aploneura lentisci</i> – Root aphid	Reduced population	Schmidt, 1993
<i>Agrostis ipsilon</i> – Black cutworm	Toxic	Baldauf <i>et al.</i> , 2011
<i>Oncopeltus fasciatus</i> – Large milkweed bug	Deterrent and toxic	Johnson <i>et al.</i> , 1985; Bush <i>et al.</i> , 1993
<i>Rhopalosiphum padi</i> – Aphid	Deterrent and toxic	Johnson <i>et al.</i> , 1985; Siegel <i>et al.</i> , 1990; Bush <i>et al.</i> , 1993

(Continued)

**Table 1** (Continued)

Metabolite and insect	Effect	References
<i>Adoryphorus coulonii</i> – Red-headed cockchafer	Deterrent	Bryant <i>et al.</i> , 2010
<i>Lepidogryllus</i> spp. – Mottled field cricket	Deterrent	Barker <i>et al.</i> , 2015b
<i>Schizaphis graminum</i> – Aphid	Toxic	Johnson <i>et al.</i> , 1985; Siegel <i>et al.</i> , 1990
<i>Teleogryllus commodus</i> – Black field cricket	Deterrent	Barker <i>et al.</i> , 2015b
<i>Spodoptero frugiperda</i> – Fall armyworm	Deterrent and toxic	Riedell <i>et al.</i> , 1991
<i>Trigonotylus caelestialium</i> – Rice leaf bug	Resistance	Shiba and Sugawara, 2009
<i>Ostrinia nubilalis</i> – European corn borer larvae	Toxic	Riedell <i>et al.</i> , 1991
<i>Wiseana cervinata</i> – Porina	Deterrent	Popay and Lane, 2000
<i>Popilla japonica</i> – Japanese beetle larvae	Toxic	Patterson <i>et al.</i> , 1991
<i>Pratylenchus scribneri</i> – Lesion nematode	Toxic	Bacetty <i>et al.</i> , 2009

### 6.1.1 Fescue foot

The restriction in peripheral blood in the extremities caused by ergovaline can result in lameness, and at its worst, necrosis and sloughing of hooves and tips of ears and tails (Garner and Cornell, 1978; Waller, 2009). The lameness and loss of hooves is a significant animal welfare issue. While most prominent in cattle, it may also occur in sheep and horses. It occurs in the cold temperatures of winter, a time of the year when it is typical for blood supply to the extremities to be reduced in response to the cold.

### 6.1.2 Fat necrosis

Cattle that have consumed toxic endophyte-infected tall fescue can build up a mass of necrotic fat (liptomatosis) in the abdominal cavity. This mass can become so large that it restricts internal organs causing digestive problems and reduced reproductive capacity. This phenomenon, commonly termed as bovine fat necrosis (Waller, 2009), can only be determined through post-mortem examination but contributes to the overall symptoms of fescue toxicosis (Waller, 2009). It has also been reported to be associated with fescue toxicosis in goats and deer (Wolfe *et al.*, 1998; Smith *et al.*, 2004).

### 6.1.3 *Fescue toxicosis*

Fescue toxicosis is generally a failure of livestock grazing endophyte-infected tall fescue to thrive, resulting in production losses. The ill thrift is particularly evident in cattle grazing in hot humid summer conditions in the USA, where it was commonly termed summer slump or summer syndrome. Cattle (and sheep) are intolerant of heat, with elevated body temperature (hyperthermia) and increased respiration rate. Compared with animals grazing forages free of ergot alkaloids, affected animals spend less time grazing and more time in shade and cooling off in dams and forming wallows. This inability to cope with heat can be attributed to the vaso-constrictive effects of ergovaline, disrupting thermo-regulatory functions in the animal. Ergovaline is also well known for its feeding deterrence (Caradus *et al.*, 2020). Additional signs of fescue toxicosis in cattle are excessive salivation, a rough hair coat and a general unthrifty appearance. This disease occurs most commonly in late spring, summer and autumn, but can also occur in winter.

Productivity losses are evident in poor weight gains, milk production, conception rates and birth weights. In US studies that have compared endophyte treatments within the same cultivar, growth rates of steers and lambs have been 30–100% greater in non-ergot alkaloid treatments (endophyte-free and selected endophytes) than toxic endophyte (Stuedemann and Hoveland, 1998; Bouton *et al.*, 2002; Parish *et al.*, 2003a, 2003b). For tall fescue cultivar Kentucky-31, dairy cows grazing endophyte-free grass have been shown to produce 22–31% more milk than those grazing toxic endophyte (Strahan *et al.*, 1987).

For horses, fescue toxicosis causes a similar reduction in growth rate as it does in cattle (Aiken *et al.*, 1993). However, effects on reproductive performance are much greater for horses and are a significant animal welfare issue (Cross, 2009). Effects are numerous, including low pregnancy rates, increased gestation lengths, birthing difficulty (dystocia), increased foal and mare deaths, thick and retained placentas, little or no milk production (agalactia), and foals may be weak and deformed.

### 6.2 *Ryegrass toxicity*

The clinical disorder referred to as ryegrass staggers occurs when livestock graze perennial and long-term hybrid ryegrasses (*Lolium boucheanum* syn. *L. hybridum*) infected with what is typically referred to as common-toxic, standard or wild-type strains of *E. festucae* var. *lolii*. Ryegrasses with common-toxic endophytes are widely naturalised in Australia and New Zealand, where ryegrass staggers have gained so much prominence. The disorder is neuromuscular and is characterised by tremors, staggering gait, convulsions and collapse, all of

which are worsened by exercise (di Menna *et al.*, 2012). It has been recorded to occur in both ruminants and monogastrics, including horses, sheep, cattle, donkeys, deer, goats, llamas, camels, alpacas and rhinoceri, in countries from all around the world. Of the tremorgenic indole-diterpenes produced *in-planta*, lolitrem B is the primary causative secondary metabolite of ryegrass staggers (Gallagher *et al.*, 1984). When livestock are removed from toxic pastures, recovery is rapid (di Menna *et al.*, 2012).

The common-toxic strains of ryegrass endophyte also produce ergot alkaloids, particularly ergovaline. Ergovaline in tall fescue causes fescue toxicosis/summer slump. A similar effect is seen in ryegrass, where the common-toxic endophyte is considered to be a significant contributor to 'summer/autumn ill thrift' (Fletcher, 1999).

Concentrations of lolitrem B and ergovaline are highly seasonally dependent, being greatest in the warmer months of the year (Watson *et al.*, 1999; Moate *et al.*, 2012; Thom *et al.*, 2013). They are closely associated with the mass of *Epichloë* mycelia, so are at greatest concentrations in the base of the pasture and in the reproductive tiller (Realini *et al.*, 2024). Concentrations become toxic to livestock from late spring as reproductive tillers rapidly elongate, and staggers can occur. In summer and autumn, pasture secondary metabolite concentrations are at their greatest and consequently most toxic, particularly in dry conditions and when closely grazed.

Along with ryegrass staggers, losses occur through reduced milk production in dairy cows and lower liveweight gain is achieved in young stock, relative to livestock grazing ryegrass that is endophyte free or infected with a selected endophyte (Fletcher, 1999; Bluett *et al.*, 2005). Sheep have faeces with higher moisture contents which leads to greater faecal soiling in the breech area (termed 'dags'), and as a consequence, a higher incidence of myiasis (flystrike). Reproductive performance in sheep can be compromised, plasma prolactin levels reduced, body temperatures and respiration rates are elevated under warm humid conditions, and there is greater mortality in sheep and cattle (Foot *et al.*, 1988; Fletcher, 1999; Fletcher *et al.*, 1999; Reed *et al.*, 2005).

### **6.3 Secondary metabolite thresholds to guide use in commerce**

For clinical toxicity, secondary metabolite thresholds have been established through dosing experiments and/or through a series of reported cases on farms (Table 2). Results can vary between studies, so ranges rather than specific threshold values are referred to. This may reflect the influence of environment, a range in susceptibility of individual animals and/or breed differences, height of grazing and grazing compared with indoor feeding studies. The values for camels, alpaca and white rhinoceri are based on a single study or observation and indicate these species are more sensitive than cattle and sheep, at least

**Table 2** For clinical toxicity, threshold concentration values of endophyte secondary metabolites produced by endophyte-infected tall fescue (ergovaline) and perennial ryegrass (ergovaline and lolitrem B) when fed to horses, cattle, sheep, camels, rhinoceri and alpaca

Animal species	Threshold concentration range in forage (ppm)	
	Ergovaline	Lolitrem B
Horse	0.30–0.50 <sup>a</sup>	0.8–1.2
Cattle	0.40–0.75	1.8–2.0 <sup>b</sup>
Sheep	0.50–0.80	1.8–2.5
Camel	nd <sup>c</sup>	≤1.1
White rhinoceros	nd	<1.0
Alpaca	nd	0.9

Compiled from di Menna *et al.* (1992), Tor-Agbidye *et al.* (2001), Aldrich-Markham *et al.* (2003), Fink-Gremmels (2005), Alabdouli *et al.* (2014), Reed *et al.* (2010) and Bluett *et al.* (2004).

<sup>a</sup>Threshold is zero for mares in late pregnancy.

<sup>b</sup>Threshold toxicity could be lower for lolitrem B when fed over an extended period (Durringer *et al.*, 2021).

<sup>c</sup>Not determined.

for lolitrem B. It is generally accepted that deer as a species are sensitive to endophyte in ryegrass, particularly Wapiti deer (*Cervus elaphus manitobensis*) (Prestidge, 1993). For endophyte-infected perennial ryegrass, there is no evidence that the production of two mammalian toxins (ergovaline and lolitrem B) interact and worsen toxicity, at least for sheep (Finch *et al.*, 2018).

While not absolute, the thresholds of Table 2 have been deployed in commerce. They have been particularly useful in determining if conserved endophytic hay and straw can be 'safely' used to feed livestock. This has been applied in Europe to hay and in Oregon, USA, for seed production straw exported to Asia (Fink-Gremmels, 2005; Craig, 2009).

While much focus has been put on thresholds for clinical toxicity, research has shown that in general the subclinical impacts increase in a linear manner as secondary metabolite concentrations increase, with no threshold (Crawford Jr. *et al.*, 1989; Schmidt and Osborn, 1993; Fletcher *et al.*, 1999; Layton *et al.*, 2004). For each 10 percentage units increase in endophyte infection in pastures, 45 g/day/animal in cattle and up to 10 g/day/animal in sheep of potential liveweight gain are lost in the USA for tall fescue and in New Zealand for ryegrass, respectively. Economic thresholds have not been defined but can be calculated on animal production losses and costs of upgrading to a safe pasture.

## 7 Tall fescue endophyte toxicity across the globe

The toxicity of endophyte-infected tall fescue has been reported across the globe, with its greatest impact being in North and South America (Table 3) (Young *et al.*, 2013).

**Table 3** *Epichloë* endophyte toxicities reported for livestock on farms globally for tall fescue

Country	Toxicity syndrome	Impacts	Livestock affected	Annual costs	Comments	Reference(s)
USA	Fescue toxicosis, fescue foot, bovine fat necrosis	Lower liveweight gain and milk production, loss of extremities, heat stress, reproductive losses	Beef cattle, dairy cows, horses	US\$1 B	Fescue toxicosis has by far the greatest economic impact	Aiken and Strickland (2013), Young et al. (2013), Ball et al. (2019)
Chile, Argentina and Uruguay	Fescue toxicosis, fescue foot	Lower weight gain, excitement, hyperaesthesia, elevated body temperature, lameness, loss of distal parts of limbs	Dairy cows, cattle	US\$54 M in Argentina	Economic impact only assessed for Argentina	Sepulveda et al. (1996), De Battista et al. (1997), De Battista (2005), Petigrosso et al. (2013)
Australia and New Zealand	Fescue toxicosis, fescue foot	Heat stress, weight loss, lameness, loss of distal parts of limbs	Cattle	- <sup>1</sup>		Cunningham (1948), Pulsford (1950), Easton et al. (1994), Hume and Sewell (2014), Reed (2014)
South Africa	Fescue foot	Lameness, necrosis of the lower tail	Cattle	- <sup>1</sup>	Along with fescue foot, suspected case of fescue toxicosis in dairy cows; may be exacerbated by ergot ( <i>Claviceps purpurea</i> ) consumption	Botha et al. (2004)
Japan	Fescue toxicosis, fescue foot	Weight loss, lameness, abortions	Cattle	- <sup>1</sup>	In cattle-fed straw imported from USA	Tor-Agbyde et al. (2001), Craig (2009), Young III and Silberstein (2012), Craig et al. (2014).
France	Fescue toxicosis, fescue foot	Reduced milk production, necrosis of tails and feet	Cattle	- <sup>1</sup>	Cases reported in France, with unconfirmed cases in Spain	Raynal (1991), Le Bars and Le Bars (1996), Bony and Delatour (2001), Bony et al. (2001)

<sup>1</sup>No estimates made of losses but are now likely to be very low given the very low prevalence of toxic tall fescues in pastures or conserved forage, and the use of selected animal-safe endophyte-infected cultivars in some markets.

## 7.1 USA

Tall fescue endophyte toxicity is most well-studied, quantified and of greatest impact in the USA. There are approximately 14 million ha of tall fescue in the USA, predominately in the east where it is referred to as the 'fescue belt' due to its dominance in grasslands, turf and roadsides (Young *et al.*, 2013; Ball *et al.*, 2019). Endophyte is needed for tall fescue pasture persistence in the southern half of the fescue belt and it is mainly livestock-toxic endemic endophytes from the widely sown cultivar Kentucky 31 and similar cultivars developed from it.

Due to the widespread occurrence of toxic fescue, and the large effects on livestock, estimates made over a decade ago place the total costs of toxic endophyte to the USA forage-based livestock industry at over US\$1 billion/year (Aiken and Strickland, 2013). It is not surprising that tall fescue endophyte toxicity has been considered the most important grass-induced toxicity in the country (Cheeke, 1995). Much of this loss occurs in the beef cattle industry, through reduced calf numbers and growth rates, with fescue toxicosis having the greatest economic impact of the three forms of toxicity (Table 3). It is generally accepted that a potential liveweight gain of 45 g/day (0.1 lb/day) is lost with each 10% increase in toxic endophyte infection in the pasture. Additional costs to the cattle industry occur through cases of fescue foot and fat necrosis. This cost has been difficult to determine, due to cattle being affected by long-term exposure, with cases not well documented or unreported (Aiken and Strickland, 2013).

In the equine sector, where ~700 000 horses are raised on endophyte-infected tall fescue, the reduced reproductive performance of mares is by far the greatest economic impact and a major welfare concern (Cross, 2009). Milk production in dairy cows is also impacted, with one study reporting a 20% loss in production for cows grazing toxic endophyte-infected tall fescue compared with endophyte-free tall fescue (Strahan *et al.*, 1987).

## 7.2 South America

Clinical and sub-clinical toxicity of tall fescue endophytes has also been documented in southern South America. Endophyte infection is widespread in tall fescue pastures and seed lots in Chile, Argentina and Uruguay. Fescue toxicosis and fescue foot have been reported for cattle and have been of greatest concern in Argentina and Uruguay due to large areas sown and/or high reliance on tall fescue grasslands. In Argentina, estimated annual losses in 1995 were US\$54 million due to lower weight gains in cattle.

Old pastures in South America are of the greatest concern, often having been sown with cultivar Kentucky 31 sourced from the USA. The frequency of toxicity has varied over the decades and was greatest in the 1980s. Compared

to the USA, the lower prevalence and impact of the tall fescue endophyte on livestock production systems has been attributed to several factors. These include greater farmer awareness of the problem, greater contents of legumes that dilute the intake of endophyte toxins, a faster rate of pasture renewal, regulation of endophyte levels in certified seed in Argentina and the use of locally bred cultivars that are now predominately endophyte free.

### 7.3 Beyond the Americas

Outside of the Americas, reports of endophyte toxicity are few and now of minor significance for livestock production systems.

In Australia and New Zealand, the widely naturalised populations of tall fescue are highly infected with toxic *Epichloë* in the temperate 'high rainfall' pasture zones. Its toxicity was renowned, which in some cases when it invaded pastures, earned it the reputation as a weed rather than a valuable pasture grass. At least in New Zealand, local naturalised 'roadside' tall fescue has been recorded with at least double the ergovaline concentrations of Kentucky 31 infected with toxic endophyte, therefore increasing the severity of the toxicosis (Easton *et al.*, 1994; Christensen *et al.*, 1998). As in South America, some paddocks in Australia had been sown in the past to cultivar Kentucky 31 which would also have contributed to toxicity.

Like Australia/New Zealand, tall fescue in South Africa is widely naturalised; however its endophyte content has not been assessed. The single case reported of endophyte toxicity was for a sown pasture and the source of the seed was not described.

Cases of endophyte toxicity in cattle in Japan have been the result of importing large volumes of endophytic seed production straw from Oregon, USA. Straw has also been sent to other southeast Asian countries but no endophyte toxicities have been reported. Through establishing secondary metabolite toxicity thresholds and secondary metabolite testing of tall fescue (and ryegrass) straw that is destined for export, the occurrence of clinical cases of toxicity in Japan has greatly diminished.

Despite being a centre of origin for tall fescue and the source of *New World* endophyte toxicity, reports of tall fescue endophyte toxicity in commerce in Europe are rare. Various studies have shown ergovaline to be at or above toxic threshold concentrations on occasions (Bony and Delatour, 2001; Bony *et al.*, 2001; Vázquez-de-Aldana *et al.*, 2001; Oliveira *et al.*, 2003; Vázquez-de-Aldana *et al.*, 2003; Zabalgogezcoa and Bony, 2005), but even in experimental conditions, it has been difficult at times to demonstrate clear effects (Emile *et al.*, 2000). Endophyte infection in cultivars is highly variable (Latch *et al.*, 1987; Vázquez-de-Aldana *et al.*, 2001). The species diversity of old pastures, and the lack of endophyte in modern cultivars, are likely to contribute to the

low toxicity. In the UK, cultivars on the recommended National Variety List must have  $\leq 5\%$  endophyte, and seed from New Zealand, Australia, South America and the USA is routinely tested for endophyte (Anon., 2023a).

## 8 Ryegrass endophyte toxicity across the globe

Reports of toxicity of endophyte-infected perennial ryegrass (Table 4) occur in the same regions of the world as do tall fescue endophyte toxicities (Table 3). However, as these grasses are best adapted to different climatic zones, and endophyte infection is variable across the world, the prevalence of toxicity is not equivalent for both species in all regions (Young *et al.*, 2013).

### 8.1 New Zealand and Australia

Globally, the impacts of perennial ryegrass endophyte toxicity have been greatest in New Zealand and the 'high rainfall' (i.e.  $>650$  mm/year) zone of south-eastern Australia (Reed *et al.*, 2005; Johnson *et al.*, 2013). Perennial ryegrass is well adapted to these climates and soils, being a commonly sown pasture grass and widely naturalised in the landscape. Old ryegrass pastures are infected at high frequencies with toxic *Epichloë*, and locally-adapted cultivars were generally infected with toxic endophyte prior to the use, some 30 years ago, of selected endophytes (Reed *et al.*, 2000; Reed, 2014; Caradus *et al.*, 2023). In many districts, endophyte-infected seed needs to be sown to ensure pasture productivity and persistence.

In these countries, a range of toxic endophyte effects have been identified and well-characterised. The first pivotal study was in New Zealand, where the link was made between ryegrass staggers in sheep and endophyte-infected ryegrass (Fletcher and Harvey, 1981). Ryegrass staggers had been a significant animal disorder that researchers had been investigating for many decades and had failed to identify the cause (Hume *et al.*, 2020). In further research, it became abundantly clear that the toxic endophytes were responsible for a range of negative impacts on livestock, beyond clinical cases of ryegrass staggers. Some of these effects mirrored those of the ergovaline-driven fescue toxicosis seen in the USA for tall fescue, so ergovaline became a key component of the research. In New Zealand, toxic endophyte is considered to be a significant contributor to 'summer/autumn ill thrift', as it is in Australia, where the combined impacts are termed 'perennial ryegrass toxicosis (PRGT)'.

As described above, reduced reproductive performance, dags, flystrike, heat stress and deaths are greater for sheep grazing toxic endophyte ryegrass pastures than ryegrass free of endophyte or infected with a selected endophyte. The major economic impact is subclinical effects on production, with reduced weight gain in livestock ( $\sim 17\%$  for sheep) and milk production

**Table 4** *Epichloë* endophyte toxicities reported for livestock on farms globally for perennial ryegrass

Country	Toxicity syndrome	Impacts	Livestock affected	Annual costs	Comments	Reference(s)
USA	Ryegrass staggers	Staggers, deaths	Cattle, sheep	- <sup>1</sup>		Galey <i>et al.</i> (1991), Galey <i>et al.</i> (1993), Tor-Agbidye <i>et al.</i> (2001), Fisher <i>et al.</i> (2004)
Chile, Argentina	Ryegrass staggers	Staggers	Dairy cows, cattle	- <sup>1</sup>	In addition to staggers, indirect evidence for grazing avoidance, reduction in forage intake and drop in milk production in dairy cows	Odrizola <i>et al.</i> (1993), Butendieck <i>et al.</i> (1994), De Battista <i>et al.</i> (1997)
Australia and New Zealand	Ryegrass staggers, sub-clinical toxicoses	Staggers, reduced milk and meat production, flystrike, faecal soiling, deaths, heat stress, reproductive losses	Dairy cows, cattle, sheep, horses, deer, alpaca	Australia: AU\$100 M. NZ: 16–18% \$ losses for sheep farms; 9% less dairy cow milk solids production	As well as ryegrass staggers in farmed animals, white rhinoceri in a New Zealand zoo have suffered staggers when fed hay	Valentine <i>et al.</i> (1993), Fletcher (1999), Fletcher <i>et al.</i> (1999), Watson <i>et al.</i> (1999), Lean (2001), Bluett <i>et al.</i> (2004), Bluett <i>et al.</i> (2005), Reed <i>et al.</i> (2005), Reed <i>et al.</i> (2010), Reed <i>et al.</i> (2011), Kellerman <i>et al.</i> (1993)
South Africa	Ryegrass staggers	Staggers	Cattle, sheep	- <sup>1</sup>		
Japan	Ryegrass staggers	Staggers	Cattle, horses	- <sup>1</sup>		Miyazaki <i>et al.</i> (2001), Miyazaki (2003), Miyazaki <i>et al.</i> (2004), Craig (2009), Young III and Silberstein (2012), Craig <i>et al.</i> (2014)
UK and Europe	Ryegrass staggers	Staggers	Cattle, sheep, horses, alpaca	- <sup>1</sup>		Lewis (1997), Holmes <i>et al.</i> (1999), Bony and Delatour (2001), Zabalgoeazcoa and Bony (2005), di Menna <i>et al.</i> (2012)

<sup>1</sup>No estimates made of losses but are likely to be very low given the generally low prevalence of toxic ryegrass in pastures or conserved forage, and the use of selected animal-safe endophyte-infected cultivars in some markets.

in dairy cows (9%). However, in Australia when PRGT is at national epidemic levels, deaths of sheep in particular can be significant, exceeding 100,000 in some years.

## **8.2 The Americas, South Africa and Japan**

Ryegrass staggers are the only ryegrass endophyte toxicity reported in the Americas, South Africa and Japan, and is infrequent.

In the USA, most pasture ryegrass cultivars are endophyte free and despite being adapted to large areas in the north, perennial ryegrass use is low, particularly relative to that of tall fescue (Young *et al.*, 2013). Coastal northern California is the only region where ryegrass staggers have been reported in livestock grazing pastures, on several properties and years. One case has been reported for sheep on pasture in Oregon. High volumes of seed production straw from Oregon of endophyte-infected turf ryegrass cultivars do represent a significant risk of toxicoses when fed to livestock within the USA or when exported (Alabdouli *et al.*, 2014; Craig *et al.*, 2014).

Of the countries in South America, only Chile and Argentina have significant areas of perennial ryegrass. In these two countries, endophyte infection is common but variable in frequency of infection in pastures and seed lots. Occurrence of ryegrass staggers is infrequent and while not proven, subclinical toxicity is likely. In South Africa just a single case of staggers has been reported on a perennial ryegrass pasture. In both South America and South Africa, some cases have been linked to seeds imported from New Zealand.

Ryegrass staggers have been reported in Japan for straw imported from the seed production fields of Oregon, USA, where the sensitivity of Japanese Black cattle may be greater than other breeds of cattle (Miyazaki *et al.*, 2001). As is the case for tall fescue straw, the occurrence of clinical cases of toxicity diminished greatly once secondary metabolite toxicity thresholds were determined and secondary metabolite testing of exported ryegrass straw commenced.

## **8.3 UK and Europe**

Endophyte has been detected in wild perennial ryegrass populations from many but not all countries in UK/Europe and at varying rates (1–100%) (Lewis and Clements, 1986; Latch *et al.*, 1987; Ribeiro *et al.*, 1996; Lewis *et al.*, 1997; Leyronas and Raynal, 2001; Jensen and Roulund, 2004; König *et al.*, 2017). While ryegrass staggers are not considered a major problem, it has been widely reported over many decades to occur in a number of countries in UK/Europe (di Menna *et al.*, 2012). This is in keeping with UK/European endophyte-infected ryegrass being the source of endophyte toxicity in the *New World*. As occurs for ergovaline in endophytic tall fescue, lolitrem B in various European

studies has shown at times to be at or above toxic threshold concentrations (Bony and Delatour, 2001; Bony *et al.*, 2001).

For the UK, as an example, a reminder about ryegrass staggers was published for veterinarians in 1995 (Pritchard and Lewis, 1995). This listed 11 counties in England and southern Wales, where ryegrass staggers had been reported, and described a surge of cases in drought years. This is despite ryegrass cultivars being largely free of endophyte and the introduction of regulations for endophyte in the UK National List agronomic testing. These regulations limit the endophyte content of new ryegrass cultivars to a maximum of 5% (Anon, 2023a). In addition, there is specific endophyte testing for cultivars originating from the Americas, Australia and New Zealand. Ryegrass is however commonly endophyte-infected in old pastures, and while being only a minor component of the sward, outbreaks of ryegrass staggers occur in drought years (Lewis and Clements, 1986; Pritchard and Lewis, 1995).

In France, endophyte frequency in pastures is highly correlated with the level of summer drought, with highly infected populations being mostly in the Mediterranean regions (Lewis *et al.*, 1997; Leyronas and Raynal, 2001). Ryegrass staggers have been reported in The Netherlands, Germany, Belgium and France (see reviews by Bony and Delatour (2001), di Menna *et al.* (2012), Lewis (1997) and Zabalgogezcoa and Bony (2005)). As seen in the UK, cases were associated with drought years, feeding of hay and the use of New Zealand-sourced seed. Highly endophyte-infected straw from turf cultivars was involved in many cases in The Netherlands and France.

The relatively low level of reported toxicity is influenced by the use of cultivars that are largely free of endophyte, the climatic conditions that influence secondary metabolite production being mostly moderate, generally low-to-moderate rates of infection in old pastures and ryegrass often being a minor component in botanically diverse permanent pastures. In addition, the *Epichloë* strain diversity that has been utilised for selected endophytes in New Zealand and Australia, with their low mammalian-toxic secondary metabolite profiles, have been derived from these European pastures (see Oliveira *et al.*, (2003); Vikuk *et al.*, (2019) for examples of lolitrem B-free or ergovaline-free endophyte-infected ryegrasses). The factors that determine toxicity might change under the rapidly changing climate associated with global warming, resulting in greater risks of toxicity in the future (McCulley *et al.*, 2014; König *et al.*, 2017; Vikuk *et al.*, 2019).

## 9 Livestock toxicity for other *Epichloë*-infected grasses

*Epichloë*-driven toxicity in livestock is not confined to ryegrass and tall fescue, with reports of toxicity for grass-endophyte associations native to southern Africa, Australia, China/Mongolia, USA and Argentina (Table 5) (Hume *et al.*,

**Table 5** Intoxication and/or narcosis of animals consuming asexual *Epichloë*-infected grasses other than ryegrass and tall fescue

Local name for toxicity/grass or English translation (country)	Grass – <i>Epichloë</i>	Livestock affected	Toxicosis	Endophyte chemistry of relevance to livestock toxicity
Intoxication				
Dronkgras (South Africa and Lesotho) <sup>1</sup>	<i>Melica decumbens</i> – <i>E. melicicola</i>	Cattle, horses, donkeys, sheep	Intoxicated, narcotic	Indole diterpenes
Staggers-like (Australia)	<i>Echinopogon ovatus</i> – <i>E. aotearoae</i> , <i>E. australiensis</i>	Cattle, sheep, goats, turkeys	Staggers, weight loss, deaths	Indole diterpenes <sup>2</sup>
Huecú or tembaldera (Argentina) <sup>3</sup>	<i>Festuca</i> and <i>Poa</i> spp. – <i>Epichloë tembladera</i>	Cattle, horses, sheep	Drunk, tremble	Indole-diterpenes. Ergot alkaloids
Narcosis				
Sleepy grass (SW USA) <sup>4</sup>	<i>Achnatherum robustum</i> – <i>E. funkii</i> and another <i>Epichloë</i> species	Cattle, horses, sheep	Narcosis, elevated body temperature	Ergot alkaloids (including lysergic acid amide), Indole diterpenes
Intoxication and narcosis				
Drunken horse grass (NW China and Mongolia) <sup>5</sup>	<i>Achnatherum inebrians</i> – <i>E. gansuensis</i> , <i>E. inebrians</i>	Cattle, horses, sheep, donkeys, mules, goats, rabbits	Intoxication, narcosis, panting, loss of tails and hooves	Ergot alkaloids (including lysergic acid amide), Stipatoxin

<sup>1</sup> Gibbs Russell and Ellis (1982), Miles et al. (1995a) and Hoare (2014).<sup>2</sup> Only *E. australiensis* has been shown to produce indole diterpenes (Seddon and Carne, 1926; Miles et al., 1998; Moon et al., 2002).<sup>3</sup> Secondary metabolite profile varies with grass species. Pomilio et al. (1989), Miles et al. (1995b), Cabral et al. (1999), Iannone et al. (2011) and Iannone et al. (2012).<sup>4</sup> Petroski et al. (1992), Jones et al. (2000), Faeth et al. (2006), Moon et al. (2007) and Shymanovich et al. (2015).<sup>5</sup> *E. gansuensis* produces indole diterpenes and *E. inebrians* produces ergot alkaloids. Dang et al. (1992), Miles et al. (1996), Schardl et al. (2013a), Chen et al. (2015) and Liang et al. (2017).

2016). Despite a diversity of grass host and *Epichloë* species, and irrespective of location in the world, several of these grass-endophyte associations cause symptoms of intoxication (drunken behaviour). Symptoms are similar to the staggers that can occur in *Epichloë*-infected ryegrass, and isolated tremorgens are implicated. In addition, a narcosis effect is reported for some grass-endophyte associations, and these are associated with the presence of ergot alkaloids. For some associations, both intoxication and narcosis are reported. Variable symptoms or occurrence of toxicity may be a result of more than one *Epichloë* species being involved or strain variation within a species.

For dronkgras in Africa and drunken horse grass in Asia, overgrazing of grasslands has been cited as a reason, amongst others, for their increased prevalence in the landscape. For drunken horse grass, this has resulted in large areas (>0.5 million hectares) that are dominated by toxic, endophyte-infected plants, being up to 100% cover in some regions. However, toxicoses are infrequent, as livestock in these areas generally show a strong avoidance of these infected grasses, and this is also the case for sleepy grass in the USA. However, the grazing avoidance results in economic impacts on the overall livestock production system, particularly so in China and to some extent in the USA.

The livestock toxicities reported to date occur only for grasses that are infected with asexual *Epichloë*, with none reported for sexual *Epichloë*. This may be somewhat surprising, as sexual *Epichloë* produce the same classes of secondary metabolites which include the mammalian toxic indole diterpenes and ergot alkaloids (Leuchtman *et al.*, 2000; Schardl *et al.*, 2013b). However, asexual *Epichloë*-grass associations in general have more secondary metabolites and in higher concentrations (Leuchtman *et al.*, 2000; Schardl *et al.*, 2012). This is consistent with the defence mutualism theory, where asexual *Epichloë* need to provide as many protective properties to the host as possible (e.g. via secondary metabolite production), as the endophyte is totally reliant on the survival of the host and spread via the host seed. Sexual endophytes can achieve spread via horizontal transmission, with less secondary metabolite production lowering any metabolic cost for the host.

As an example, for a range of sexual and asexual *Epichloë*-infected grasses in Spanish dehesa grasslands, ergovaline was detected in four *Festuca* species and *Holcus lanatus* and was absent in another four grass species (Vázquez-de-Aldana *et al.*, 2003). The fine fescues *F. ovina* and *F. rubra* infected with sexual *E. festucae* had a five-fold lower concentration of ergovaline than tall fescue infected with asexual *E. coenophiala*. There is an absence of clinical signs of ergot alkaloid toxicity in cattle grazing these grasslands. This is despite the high potential for toxicity, as *F. rubra* is a common species and highly infected with endophyte. The lack of toxicity is attributed to the high plant species diversity of these grasslands, diluting any mammalian toxic endophyte secondary

metabolites in the animal's diet (Zabalgogea et al., 1999; Vázquez-de-Aldana et al., 2003). Grazing avoidance due to deterrence caused by ergovaline may also play a role (Bazely et al., 1997).

## 10 Reducing toxicity to livestock

In regions of the world where toxicity of *Epichloë* to livestock is a significant concern, several options exist for farmers to minimise or eliminate adverse effects on livestock for toxic tall fescue and ryegrass pastures. These have been well reviewed, mostly for tall fescue endophyte toxicity, and include the novel use of selected endophyte strains for tall fescue and ryegrass (Ball et al., 1993; Roberts and Andrae, 2004; Aiken and Strickland, 2013; Young et al., 2013; Caradus et al., 2021a; Poudel et al., 2023; Bastías et al., 2024). The options range from total avoidance of *Epichloë* toxins to dilution of toxins in the diet, pasture/grazing management to limit toxin intake and the use of selected strains of *Epichloë*. Each has advantages and disadvantages. In Australia, New Zealand and USA, where endophyte underpins pasture productivity, trade-offs may occur when maximising the pasture performance benefits of *Epichloë* while minimising or eliminating the detrimental effects on livestock.

### 10.1 Forages free of *Epichloë* mammalian toxins

As the production of mammalian toxins is a trait of the *Epichloë* endophyte, endophyte-free cultivars of tall fescue and ryegrass can be utilised on farms with confidence that livestock will thrive. These cultivars can be used in regions where endophyte is only occasionally needed to boost pasture performance, although this may be hard to predict, particularly in the face of climate change and the occurrence of new pasture pests through biosecurity breaches. In addition, endophyte-free cultivars may be utilised in farming systems where pastures are planned to be short-term e.g. in a mixed livestock/cropping systems and/or where practices such as irrigation may lessen stresses on pastures.

There is a range of non-*Epichloë* grass species and other forage species (legumes, herbs and brassicas) that can be utilised as alternative forages or as conserved feed, particularly during periods of the year when *Epichloë*-infected pastures are most toxic. Each of these species has their own set of limitations which may include restricted area of adaption, risk of other livestock disorders and difficulties in establishment and grazing management, all of which limit their application and widespread adoption.

In Argentina, Iannone et al. (2011) also highlighted the prospects of using grass species that are infected with asexual *Epichloë* where no toxic effects

for cattle are known. In this region, some of the endophytic *Bromus* and *Poa* species are known for their forage quality. In addition, some strains of *Epichloë* infecting tall fescue and ryegrass are livestock safe and these are discussed in the section 'Selected Endophytes'.

## 10.2 Dilution of endophyte toxins

Various species can be used in mixed species pastures to dilute the concentration of *Epichloë* mammalian toxins in the forage that is consumed by livestock while grazing. This may include non-endophytic grasses, legumes and herbs. Of these, the use of clovers (*Trifolium* spp.) in mixed pastures has received the greatest attention, as they are highly valued for their ability to boost animal performance through high forage quality and for the addition of atmospheric nitrogen (N) to the soil through N-fixation in clover roots.

In the USA, the addition of perennial clovers (white and red; *T. repens* and *T. pratense*, respectively) to toxic endophyte tall fescue pastures has been widely promoted as a tool to dilute and so alleviate the toxicosis (Roberts and Andrae, 2004). In southern South America, the widespread use of legumes in pastures is considered one of the factors that has reduced the economic impact of tall fescue endophyte toxicity in the region (De Battista *et al.*, 1997). In Australia and New Zealand, ryegrass swards commonly have subterranean (*T. subterraneum*) and/or white clovers, with limited use of red clover. Cosgrove *et al.* (1996) found that under severe ryegrass-endophyte challenge, white clover reduced the severity of ryegrass staggers in cattle in central New Zealand and advocated for high clover contents in swards, along with other management factors. However, it can be difficult to maintain sufficiently high contents of white clover in endophytic ryegrass swards, as endophyte-infected perennial ryegrass is known to suppress clovers (Foot *et al.*, 1988; Sutherland *et al.*, 1999). In addition, the dry hot conditions in summer/autumn that increase the risks of toxicity are unfavourable for white clover, and as an annual species, subterranean clover is not present. Recently, Poudel *et al.* (2023) have postulated that condensed tannins produced by some legumes could be usefully deployed to reduce tall fescue toxicity.

Another form of dilution is to breed for low secondary metabolite producing grass–endophyte associations. This has been progressed by selecting lower ergovaline concentrations within mammalian-toxic endophyte-infected 'Jesup' tall fescue. Selection proved highly effective with high heritability (Adcock *et al.*, 1997). However, tall fescue persistence of the low ergovaline selection was reduced relative to unselected mammalian-toxic endophyte-infected tall fescue, and weight gains of lambs were also lower than endophyte free (Bouton, 2000; Bouton and Hopkins, 2003).

### 10.3 On-farm management

Grazing management has received the greatest attention of the various strategies that farmers can deploy to lessen the concentrations of secondary metabolites in the diets of livestock. For ryegrass in New Zealand, rapid rotational grazing of sheep, avoiding grazing to low residuals, can be highly effective in reducing ryegrass staggers and most likely results in less subclinical toxicity. Under this management, livestock primarily consume leaf blades in the upper portion of the sward, the horizon in the pasture where toxins are of lowest concentration. It is likely that this is why toxicity on-farm is less in dairy cows than that seen in sheep (Prestidge, 1993). The same principle applies to tall fescue.

The toxicity profile of the sward changes in late spring as rapid elongation of reproductive tillers elevates seed heads, that have high concentrations of alkaloids, into the grazing horizon, therefore increasing the risk of toxicity (Rottinghaus *et al.*, 1991; Ball *et al.*, 1997a; Roberts and Andrae, 2004). This is particularly so if seedheads are selectively grazed by livestock (Aiken and Strickland, 2013). This risk can be reduced by mowing ('topping') reproductive tillers, or close grazing or silage making in the very early stage of elongation, being aware that lolitrem B and ergovaline are present in silage (and hay) and this may take some time to decline to safe levels or require ammoniation treatment (Roberts and Andrae, 2004; Fink-Gremmels, 2005; Fletcher, 2005). Research in the USA has shown that tall fescue seedheads can be suppressed chemically, and severity of toxicity in cattle reduced, through application of plant growth regulators or herbicides (Roberts and Andrae, 2004; Aiken and Strickland, 2013). In addition, these chemicals can improve forage quality, such as digestibility, but they also pose risks to tall fescue and non-toxic companion species by hindering growth leading to decreased forage availability.

### 10.4 Selected endophytes

In nature, it is common to find a dominant endophyte strain within an *Epichloë* species. This is not surprising when certain secondary metabolite profiles provide a selective advantage to the grass–endophyte association (Schardl *et al.*, 2012). However, where researchers have collected many endophytes, in particular, from asexual *Epichloë* species, considerable genetic and secondary metabolite diversity has been found (van Zijll de Jong *et al.*, 2008; Ekanayake *et al.*, 2012; Takach and Young, 2014; Kaur *et al.*, 2015). In many situations, strains have been discovered that lack some of the metabolites that are problematic for animal health but express metabolites which provide insect pest tolerance. These secondary metabolite profiles are confirmed by extensive chemical analysis of grass–endophyte associations, along with gene studies of

*Epichloë* to determine the absence/presence and/or mutation of genes in the biosynthetic pathways (Young *et al.*, 2013).

This diversity has been capitalised on by selecting *Epichloë* strains with favourable secondary metabolite profiles and transferring them, usually by artificial inoculation in the laboratory, to elite grass cultivars to form novel host grass–endophyte associations for use on farms (Johnson *et al.*, 2013; Caradus and Johnson, 2020). This has been critical for Australia and New Zealand for perennial ryegrass, and the USA for tall fescue. In these countries, the widely naturalised populations and locally adapted cultivars were highly infected with mammalian-toxic *Epichloë* strains, with no useful strain variation identified (Simpson *et al.*, 2012; Reed, 2014; Young *et al.*, 2014; Ball *et al.*, 2019; Caradus *et al.*, 2023).

Selected endophytes are the first patented technology to be placed in grass cultivars (Bouton and Hopkins, 2003). The first release of a selected endophyte was in the early 1990s for use in perennial ryegrass (Johnson *et al.*, 2013). Since then, a number of selected strains have been deployed in tall fescue, several ryegrass species, *Festulolium* and meadow fescue (Table 6).

Some selected strains from tall fescue and ryegrass lack the mammalian toxic secondary metabolites ergovaline, lolitrem B and epoxyjanthitrems. While this ensures that the endophytes are benign for livestock, it does lead to reduced pest protection of the host grass, the possibility of overgrazing by livestock, and so poorer agronomic performance relative to cultivars infected with naturalised highly toxic endophytes. Relative to endophyte-free counterparts, selected endophytes improve the agronomic performance of cultivars (Caradus *et al.*, 2021a).

An approach to improve the agronomic efficacy of novel grass–endophyte associations for tall fescue and ryegrass is the utilisation of strains that retain the expression of lolitrem B and/or ergovaline, as these metabolites provide some level of pest protection (Caradus and Johnson, 2020). To reduce the risk of toxicity to livestock, these metabolites are at medium-to-low levels, as achieved through a combination of using certain endophyte strains and/or selecting grass–endophyte combinations for reduced metabolite concentrations as their expression is under strong plant genetic control (Easton, 2007; Faville *et al.*, 2015). This strategy does run the risk of livestock toxicity in some seasons or in adverse environments, which is predicted to occur more frequently due to climate change (Caradus and Johnson, 2020).

The discovery of strain AR37 (*Epichloë* sp. LpTG-3) for use in ryegrasses has illustrated that naturally occurring strains exist that can exceed the pest protection imparted by the common mammalian toxic strains and yet have reduced toxicity to livestock (Caradus and Johnson, 2020; Caradus *et al.*, 2021b). In New Zealand, the use of this strain has led to significant environmental and economic benefits (Caradus, 2023a).

### 10.4.1 Interspecies transfer

To improve the pest protection profile of ryegrass without compromising livestock health and performance, non-ergovaline-producing endophytes have been transferred from tall fescue and meadow fescue to enable loline expression in perennial ryegrass and Italian ryegrass (Matsukura *et al.*, 2012; Johnson *et al.*, 2019). While this interspecies transfer of *Epichloë* has been achievable, commercial application has been hindered by poor loline expression, sub-optimal transmission to seed and longevity in stored seed, highlighting the strong host specificity of *Epichloë* endophytes (Johnson *et al.*, 2019; Caradus and Johnson, 2020).

The host plant of novel endophyte–grass associations can have a major impact on the secondary metabolites produced and the biological effects induced. For example, the selected endophyte AR542 (*E. coenophiala*) combined with Mediterranean-type tall fescue, a tall fescue morphotype genetically distinct from Continental and rhizomatous types (Hand *et al.*, 2010) induced a previously unknown disease, equine fescue oedema. The same endophyte combined with Continental-type tall fescue did not (Munday *et al.*, 2017). To add to the complexity, equine fescue oedema was only observed in horses, with sheep, cows and rabbits unaffected. Although a loline analogue was initially implicated (Bourke *et al.*, 2009) this was later disproved, and the cause of equine fescue oedema is still unknown (Finch *et al.* 2017).

### 10.4.2 Persistence

The persistence of elite cultivars with selected endophytes, in comparison to tall fescue and ryegrass with their mammalian toxic endophytes, has been questioned. This is particularly so for the range of selected endophytes in perennial ryegrass, where persistence is correlated with which secondary metabolites are produced, and in some cases the concentration of those metabolites, as this largely determines the range and effectiveness of insect pest control (Tables 1 and 6).

Overgrazing of cultivars with selected endophytes, due to no or reduced expression of secondary metabolites associated with feed deterrence, can lead to reduced persistence and can be a concern in stressful conditions in the warmer months of the year (Waller, 2009; Fletcher, 2012; Aiken and Strickland, 2013; Young *et al.*, 2013; Ball *et al.*, 2019). Farmers fail to recognise overgrazing is occurring, particularly so when previous farmer experience is with the mammalian toxic wild-type endophyte-infected tall fescue or ryegrass which can self-regulate grazing intensity. The ergovaline produced by wild-type endophytes has a strong deterrent effect on livestock when they graze close to the base of the pasture where concentrations are at their highest (Caradus

**Table 6** Major end pathway secondary metabolite profiles for selected endophytes used on farms since the early 1990s for cultivars of tall fescue, ryegrasses, Festulolium and meadow fescue, in comparison to the highly mammalian toxic strains in tall fescue and perennial ryegrass. For further details see (Caradus and Johnson, 2019; Caradus and Johnson, 2020; Eady, 2021; Caradus and Hume, 2023)

Peramine	Major end pathway secondary metabolites <sup>1</sup>					Strain name <sup>2</sup>
	Ergovaline	Lolitrein B	Epoxyanthitremis	Lolines		
Original host: tall fescue <sup>3</sup>						
+	+	-	-	+		Toxic
+	-	-	-	+		AR542 (MaxQ, MaxP), AR584 (MaxQ II, MaxP), Strain 4 (ArkPlus, ArkShield), E647 (Protek)
+	+(low)	-	-	+		BE9301A (E34)
Original host: perennial ryegrass <sup>4</sup>						
+	+	+	-	-		Common toxic, wild-type, standard
+	+	-	-	-		AR5 (Endosafe, Endo5), AR6 (Endosafe)
+(low)	+(low)	+(low)	-	-		nea2 (NEA) <sup>5</sup>
+	+	-	-	-		nea3, nea6 <sup>5</sup>
+	-	-	-	-		AR1, Edge
-	-	-	+	-		AR37, CM142, NEA12 and RGT18
Original host: meadow fescue <sup>6</sup>						
-	-	-	-	+		U2, Happe, AR1017 (MaxR)

<sup>1</sup>+' = present, '-' = absent.

<sup>2</sup>Strain name, with tradename given in parentheses if different to strain name.

<sup>3</sup>Utilised in Continental-type tall fescue.

<sup>4</sup>Utilised in perennial, long and short-term hybrid, and Italian ryegrasses (*L. perenne*, *L. boucheanum* syn. *L. hybridum* and *L. multiflorum*, respectively).

<sup>5</sup>May be sold in mixtures as NEA2 (nea2 + nea6) and NEA4 (nea2 + nea3).

<sup>6</sup>Utilised in perennial ryegrass, Festulolium or meadow fescue.

*et al.*, 2020). Selected endophytes either lack or have low concentrations of ergovaline (Caradus and Johnson, 2020).

### **10.4.3 Adoption**

The development and use in commerce of selected endophytes is not surprising, for as biological control agents (BCAs), they are cost-effective, environmentally sound, enduring and highly effective at reducing biotic stresses, along with abiotic stresses in some cases. They are considered ideal BCAs, due to fungal plasticity, effective mechanism of action, secondary metabolite and genetic diversity, propagation via seed and obligate nature (Card *et al.*, 2016; Bastías *et al.*, 2024).

On-farm, there are no special techniques, equipment or new management procedures required, as the selected endophytes are already incorporated into the seed of elite grass cultivars by the plant breeding/seed industry. To minimise reinvasion of toxic endophytes, farmers need greater adherence to best management preparation of seedbeds and take simple steps to reduce reintroduction of seed with toxic endophytes as these may be more competitive than selected endophytes (Hume and Barker, 2005). In addition, greater attention to grazing residuals are needed to prevent overgrazing (as discussed earlier).

The traits that make *Epichloë* an effective BCA have also enabled the plant breeding and seed industries to achieve cost-effective product development, intellectual property protection, marketing and distribution of selected endophyte-infected grass seed products (Bouton and Hopkins, 2003; Card *et al.*, 2016; Caradus *et al.*, 2021b). This has involved the discovery and characterisation of endophytes, transfer from wild host grasses to elite grass genetics, and breeding for compatible, stable associations that provide effective bioprotective properties, as well as transmitting and storing well in seed at commercial scale (Johnson and Caradus, 2019). This has created extra complexity and costs to breeding programmes (Eady, 2021), and, in particular, the need to thoroughly test the pest protection profile and livestock safety of new grass-endophyte cultivars (Bouton and Hopkins, 2003; Thom *et al.*, 2012; Caradus *et al.*, 2021b).

While selected endophytes have some potential disadvantages, Ball *et al.* (2007) considered that for tall fescue in the USA their use is the only long-term solution for eliminating toxicity to livestock while still having the agronomic benefits of endophyte. It is a similar situation for perennial ryegrass in New Zealand and Australia (Heeswijck and McDonald, 1992; Prestidge, 1993), but greater compromises may be needed between livestock performance/health and agronomic performance. Despite this, the uptake of selected endophytes has been rapid and high in New Zealand where it is estimated that 90% of the

proprietary perennial ryegrasses are sold with selected endophytes (Caradus *et al.*, 2013, 2021b).

In contrast to New Zealand, there has been poor uptake in the USA of selected endophytes in tall fescue, despite early significant sowing of selected endophytes and numerous grazing experiments and on-farm demonstrations (Ball *et al.*, 2007). Reasons for poor uptake are numerous, including a lack of awareness of endophyte toxicity, uncertainty regarding the process of killing old and planting new pasture, high seed costs and unclear financial benefits (Roberts and Andrae, 2005; Poore *et al.*, 2019). This has driven the formation of the Alliance for Grassland Renewal (<https://grasslandrenewal.org/>), a concerted collaborative multi-organisation, multi-state effort to enhance farmer understanding and adoption of 'Novel endophyte tall fescue' (Poore *et al.*, 2019; Roberts and Andrae, 2023).

#### **10.4.4 Extending use of grasses through selected endophytes**

The value of using selected endophytes goes beyond replacing the use of mammalian toxic endophytic grasses. Selected endophytes that have no mammalian toxins can be utilised to enhance agronomic performance of pastures in regions where *Epichloë* are not currently used in specific grass species. This may become of increasing importance as climate change increases biotic and abiotic stresses on pastures (Caradus and Hume, 2023).

In Australia and New Zealand, the use of tall fescue has been very limited despite being well adapted to harsh soil and climatic conditions where perennial ryegrass struggles (Easton *et al.*, 1994). There is little occurrence of toxic endophyte-infected tall fescue in pasture and it has been well-documented that mammalian-safe endophytes can greatly improve agronomic performance of tall fescue in these regions, with no negative effects on livestock (Easton *et al.*, 1994; Hume *et al.*, 2009; Fletcher, 2012; Hume and Sewell, 2014). The greatest opportunity is in Australia, where currently it is only used in 7% of its potential adaptive area of 16.8 M ha (Young *et al.*, 2013). Opportunities also exist in other regions of the world, such as Chile, Argentina and Uruguay (De Battista *et al.*, 1997; De Battista, 2005), and possibly southern Europe.

A similar but less certain situation exists for utilising selected endophytes to extend the use of perennial ryegrass in the USA, UK/Europe and southern South America, where currently pasture ryegrass cultivars are largely endophyte free. In the USA, endophyte has been shown to reduce invertebrate pests (Young *et al.*, 2013) but clearly demonstrated agronomic efficacy in the field is lacking with only limited (reported) experimentation (Anon, 2024). In contrast, endophyte is known to enhance the performance of turf ryegrasses in the USA (Brilman, 2005; Young III and Silberstein, 2012; Stewart *et al.*, 2022). In the UK/Europe, a region where perennial ryegrass is used widely, currently there is no

clear, compelling evidence for agronomic benefit and/or control of important insect pests, based on limited experimentation. This is despite endophyte being common in wild populations and high in old pastures and drier environments implying that endophyte is advantageous for plant performance (Lewis, 2001; Zabalgoceazcoa and Bony, 2005). In southern South America, based on limited data, there appears to be no agronomic benefits of endophyte infection in perennial ryegrass (De Battista *et al.*, 1997; De Battista, 2005). However, given the agronomic advantages of endophyte infection in tall fescue in this region, there may be untapped opportunities to utilise selected endophytes to enhance perennial ryegrass pastures.

## 11 Mammalian safe *Epichloë* of annual ryegrasses and meadow fescue

Not all associations of *Epichloë* endophytes with their natural grass host have been found to be toxic to grazing animals. In particular, the two agricultural species Italian ryegrass (*L. multiflorum*) and meadow fescue (*F. pratensis*) may contain asexual endophytes which while producing some secondary metabolites are benign on livestock.

### 11.1 Italian ryegrass

*E. occultans* is an asexual seed-transmitted species found in most of the annual *Lolium* species: *L. canariense*, *L. multiflorum*, *L. persicum*, *L. remotum*, *L. rigidum*, *L. temulentum* and *L. subulatum* (Moon *et al.*, 2000; Moore *et al.*, 2015; Hume *et al.*, 2020). It is a natural allopolyploid of *E. baconii* and *E. bromicola*. Hyphae colonise meristematic tissue, but in leaves its hyphae are only found as a dense mycelial mass located at the very base of the leaf sheath (Christensen *et al.*, 2002). This contrasts with most other *Epichloë* species where hyphae are found throughout the leaf sheath, aligned to the leaf axis (Christensen *et al.*, 2002).

Lolines are produced by *E. occultans* when infecting *L. multiflorum* and several other annual *Lolium* species (Sugawara *et al.*, 2006; Moore *et al.*, 2015; Bastías *et al.*, 2019). The occurrence of lolines in *L. temulentum* was discovered many years before it was known to be associated with endophyte infection (Hofmeister, 1892). In a comparative review, Realini *et al.* (2024) found that loline concentrations in *E. occultans*-infected vegetative tissue of *L. multiflorum* and *L. rigidum* were similar to that found in both tall and meadow fescue but were only 7–13% of the concentrations in reproductive tissues of the fescues. The reduced secondary metabolites may reflect the unique hyphal distribution in the *E. occultans*-infected plants.

Loline concentrations in *E. occultans*-infected seed can be considerable, with Moore *et al.* (2015) reporting a maximum value of 2220 ppm in a *L.*

*persicum* accession. Peramine has been reported in *E. occultans*-infected inflorescences and/or seed for *L. multiflorum*, *L. persicum*, *L. rigidum* and *L. temulentum* (Sugawara *et al.*, 2006; Moore *et al.*, 2015). It is likely that lolines and peramine reduce seed predation over the crucial summer period.

In New Zealand, early seedling establishment of annual/Italian ryegrass is often enhanced by *E. occultans* infection through increased protection against Argentine stem weevil (Stewart, 1987; Hume *et al.*, 1993). Similarly, Cooper *et al.* (2007) in northern New Zealand reported that Italian ryegrass infected with *E. occultans* had advantages in plant survival and productivity of mature plants, which was attributed to reduced damage from African black beetle. In other studies, *E. occultans*-infected *L. multiflorum* has been shown to have reduced survival of rice leaf bug in Japan (Shiba and Sugawara, 2009; Shiba *et al.*, 2011) and reduced feeding damage by black cutworm in Argentina (Gundel *et al.*, 2020). In a further Argentinian experiment, *E. occultans* also reduced the impact of two out of four soil-borne pathogens at seedling establishment for *L. multiflorum* (Pérez *et al.*, 2013). Regardless of these favourable Argentinian pest and disease results, agronomic benefits of *E. occultans* are not evident in limited trialling in pastures in southern South America. This is despite the high rates of *E. occultans* infection in naturalised annual ryegrasses in this region (De Battista *et al.*, 1997; De Battista, 2005).

## 11.2 Meadow fescue

*F. pratensis* ecotypes are commonly infected with the endophyte *E. uncinata*, a hybrid of *E. bromicola* and *E. typhina*. In addition, a single occurrence has been reported in *F. pratensis* of a second endophyte *E. siegelii*, a hybrid of *E. amarillans* and *E. elymi* (Craven *et al.*, 2001). In northern Europe and the European Alps, endophyte-infected ecotype populations can be found in many countries and in cultivars at varying levels of infection (Saari *et al.*, 2009; Cagnano *et al.*, 2019). Surprisingly, the only *Epichloë*-infected meadow fescue ecotype found in the southern hemisphere does not occur in a cold temperate region but instead occurs in the subtropical north of New Zealand where insect pressure can often be severe (Cooper, 1996).

Both *E. uncinata* and *E. siegelii* produce high concentrations of loline alkaloids, usually more N-formyllooline than N-acetyllooline and N-acetylnorlooline (Patchett *et al.*, 2011b; Cagnano *et al.*, 2019). These alkaloids are not known to cause any livestock problems but are well known for providing resistance to many insects (Fletcher *et al.*, 2001; Schardl *et al.*, 2007; Caradus and Johnson, 2020). Neither *E. siegelii* nor *E. uncinata* produce peramine due to mutations in the peramine gene (Berry *et al.*, 2015).

The use of meadow fescue and meadow fescue types (Festuloliums), with their *E. uncinata* endophyte, have found a small commercial role on farms in

New Zealand due to its effects on a wide number of insect pests (Fletcher *et al.*, 2001; Bryant *et al.*, 2010; Caradus and Johnson, 2020; Caradus *et al.*, 2021b; Caradus and Hume, 2023). For *E. uncinata*-meadow fescue, it is often sown in mixtures with tall fescue with a selected endophyte. As well as insect protection, *E. uncinata* endophyte infection may increase the drought tolerance and so the competitive ability of meadow fescue in the field (Malinowski *et al.*, 1997a; Malinowski *et al.*, 1997b).

## 12 Endophyte effects on food quality and safety

As well as animal toxicity, consideration also needs to be given to the risk of endophyte-expressed compounds to human health (Miyazaki *et al.*, 2004; Shimada *et al.*, 2013). Since animals are consuming these metabolites, it is possible that they go up the food chain to be consumed by humans eating animal products. To determine the risk to human health, toxicity to monogastric animals must be determined as well as the concentrations of metabolites in food. Once this information is available, a risk assessment can be performed.

### 12.1 Toxicity of endophyte metabolites to monogastric animals

Different routes of administration can be used for the determination of toxicity using non-ruminant animals. Using intravenous (i.v.) and intraperitoneal (i.p.) administration, the compounds become immediately bioavailable, whereas compounds dosed orally must be first absorbed by the gastrointestinal tract before going through the pre-system 'first pass' of the liver. Oral administration is obviously the most relevant to human consumption, with both i.v. and i.p. dosing giving an overestimation of toxicity. Different oral dosing regimes can also be used, with acute toxicity being a one-off dose and chronic toxicity being repeated dosing.

The toxicity of ergot alkaloids has been determined. For ergotamine, an acute oral LD<sub>50</sub> for mice, rats and rabbits has been determined to be 3200, 1300 and 550 mg/kg body weight (BW), respectively (Griffith *et al.*, 1978). Chronic dosing of ergotamine, ergometrine or  $\alpha$ -ergocryptine to rats has demonstrated a no observable adverse effect level (NOAEL) of 0.22–0.60 mg/kg/day. As ergot alkaloids have been used for the treatment of headaches since the 1800s, toxicokinetic and bioactivity data are available. These studies have shown that humans have poor gastrointestinal absorption of ergot alkaloids, and following absorption, >90% is eliminated by 'first pass' metabolism. The combination of these factors results in an oral bioavailability of <1% for ergot alkaloids (Little *et al.*, 1982; Saper and Silberstein, 2006).

Using mice, the acute oral LD<sub>50</sub> of chanoclavine exceeded the limit dose specified by OECD guideline 425 (2000 mg/kg) (OECD, 2022). Chanoclavine fed as part of mouse diet, yielding a dose rate of 123 mg/kg/day, induced no adverse effects (Finch *et al.*, 2019).

The toxicity of indole-diterpenoids has also been investigated (lolitrems and epoxyjanthitrems). The lolitrem class of compounds has been assessed by incorporating pure lolitrem B into the diet of mice. The mice were fed for 90 days at an average dose rate of 0.15 mg/kg lolitrem B and showed no adverse effects (Finch *et al.*, 2022a). Additionally, a further trial demonstrated that a diet containing 1.06 ppm lolitrem B fed to two full generations of mice had no effect on gestation period, number of pups born or growth of pups (Finch *et al.*, 2022a).

Epoxyjanthitrems have been tested by incorporating endophyte-infected AR37 seed into the diet of mice. A dose rate of 3.17 mg/kg/day had no effect on mice over 90 days of feeding and a diet containing 20.1 ppm epoxyjanthitrems had no effect on mice in a two-generation reproduction study (Finch *et al.*, 2022a).

For loline alkaloids, a toxicological assessment was conducted by incorporating endophyte-infected meadow fescue seed into the diet of mice. For 3 weeks, mice ate lolines at a daily rate of 415 mg/kg with no adverse effects (Finch *et al.*, 2016). In a further trial, mice were fed seed containing high concentrations of lolines and peramine with no adverse effects (Finch *et al.*, 2022b).

## **12.2 Endophyte expressed metabolites in food**

For ergot alkaloids, dosing experiments with livestock suggest a quick clearance. The serum of steers dosed with ergolines, ergotamine or ergine by i.v. injection showed an immediate drop in toxin concentration with elimination complete within 2 hours (Moubarak *et al.*, 1996). Similarly, the i.v. administration of ergovaline to sheep showed rapid metabolism/elimination with the toxin being undetectable within 1 hour (Jausaud *et al.*, 1998). These observations suggest that residues of ergot alkaloids in animal products should be low. Consistent with this hypothesis, Schumann *et al.* (2009) were unable to detect ergot alkaloids in the milk or blood of cows fed ergot at 4.1–16.3 µg/kg BW and similarly dairy cows fed 3 µg/kg BW ergot had no ergot alkaloids in milk (Wolff *et al.*, 1995). Although below the limit of quantification, ergovaline was detectable in milk of lactating ewes consuming endophyte-infected ryegrass hay (Zbib *et al.*, 2015). In the same study, ergovaline could be detected in the liver, fat and kidney of the sheep but not in muscle or brain (Zbib *et al.*, 2015). Realini *et al.* (2005) showed ergovaline residues (2.81 ppb) in the subcutaneous fat of steers grazing endophyte-infected tall fescue.

The presence of indole-diterpenoids (lolitrem B and epoxyjanthitrems) in livestock consuming endophyte-infected ryegrass has also been investigated. In Japan, Miyazaki *et al.* (2004) showed that Japanese Black cows consuming endophyte infected ryegrass straw, and exhibiting ryegrass staggers, had lolitrem B residues in perirenal fat (210 ppb) but no residues in muscle, liver, kidney or brain. A further study conducted in the US also detected lolitrem B in the fat of Angus steers fed a diet of endophyte-infected ryegrass straw, where concentrations reached a maximum of 40 ppb (Duringer *et al.*, 2021). Similarly, monthly fat biopsies in New Zealand of sheep grazing ryegrass pastures infected with common toxic (containing lolitrem B) or AR37 (containing epoxyjanthitrems) endophytes showed maximum concentrations of 61.8 and 1032 ppb lolitrem B and epoxyjanthitrems, respectively. Toxins did not accumulate in fat, instead reflecting the concentrations being consumed at the time (Finch *et al.*, 2012). In the French study by Zbib *et al.* (2015), trace amounts of lolitrem B were detected in the milk of lactating ewes fed endophyte-infected hay. The greatest concentration of lolitrem B was found in abdominal fat (2.39 ppm) with detectable amounts in liver, kidneys and muscle, although undetectable in brain tissue. In a further New Zealand experiment, dairy cows fed endophyte infected common toxic and AR37 ryegrass pastures had toxin carryover into milk with maximum concentrations of 5 and 109 ppb for lolitrem B and epoxyjanthitrems, respectively. Concentrations in milk responded quickly to what the cows were eating and within 8 days of feeding toxin-free pastures the toxins could no longer be detected (Finch *et al.*, 2013).

### **12.3 Risk assessment**

Ergot alkaloid residues which have been observed in fat and milk are low, and this, coupled with the very low bioavailability in humans, appears to make this class of alkaloids a very low risk to human health. Since ergots contaminate grain and humans eat grain this presents a genuine safety risk. For this reason, the European Food Safety Authority (EFSA) convened committees in both 2012 and 2017 to provide scientific opinions on ergot alkaloids (EFSA, 2012, 2017). An acute reference dose (ARfD), defined as the amount that can be eaten safely within a 24-hour period, was set at 1 µg ergot alkaloids/kg bodyweight. To reach this ARfD, a 70 kg human would have to eat 24 900 kg of fat contaminated at the concentration detected by Realini *et al.* (2005) (2.81 ppb), which is physically impossible.

Unlike the ergot alkaloids neither lolitrems nor epoxyjanthitrems are associated with grain so there is no risk assessment data available. However, using the animal data and applying uncertainty factors, a human risk assessment can be completed. A safety factor of 10 is applied to account for the species

difference (mouse and human) and a further 10 is applied to account for possible variation in the human population. In the mouse toxicological assessment of lolitrem B and epoxyjanthitrems, dose rates of 0.15 and 3.17 mg/kg/day were shown to have no effect. Applying the combined 100-fold safety factors and considering a 70 kg human, a total of 0.105 and 2.22 mg lolitrem B and epoxyjanthitrems could be safely eaten in 24 hours (ARfD). Taking the highest concentrations measured in milk (Finch *et al.*, 2013) this human would have to drink 20 L of milk to reach the ARfD. Considering the highest concentrations detected in fat (Finch *et al.*, 2012), a 70 kg human would have to consume 1.7 kg and 2.2 kg of fat contaminated with lolitrem B or epoxyjanthitrems, respectively, to reach the ARfD. Assuming that meat is 20% fat then this would equate to 8.5 kg and 11 kg of meat.

### **12.4 Conclusion on impacts of endophyte metabolites on human health**

The available evidence suggests that it is highly unlikely that the endophyte metabolites lolitrem B, ergovaline and epoxyjanthitrems are a risk to human health through the consumption of animal-derived food products. Due to the presence of sclerotia (ergot) in grain they are regulated and ergot alkaloids are regulated in animal feed. In contrast, there are no regulations for indole-diterpenoids, but as new selected endophytes are commercialised in elite grass cultivars, new endophyte expressed metabolites could be introduced into human food. Without regulation, the onus is on the developers of these new endophytes to undertake toxicological evaluations to ensure food safety.

## **13 Beyond pasture grasses**

The discoveries of the effects of endophyte in ryegrass and tall fescue in pastures, and the benefits of seeking and utilising endophyte strain diversity, have spurred research into application of endophytes in turf grasses and cereals.

### **13.1 Application for turf**

As occurred with pasture grasses, in particular regions of the world such as the USA and New Zealand, breeders had been unwittingly selecting for endophyte in turf grasses before the role of *Epichloë* in pastures was discovered in the 1970s/1980s (Funk *et al.*, 1994; Stewart, 2005). In the USA, the significance of endophytes in the breeding of turf grasses was first recognised some 40 years ago (Funk *et al.*, 1983) and by the early 1990s Funk *et al.* (1993) reported 'turf-grasses have frequently shown dramatic enhancement of resistance to many

foliage-feeding insect pests'. To date the application of *Epichloë* endophyte to turf grasses has been mostly in perennial ryegrass and tall fescue. Opportunities exist for other turf species such as fine fescues (*Festuca* species) but there are significant barriers for application such as epiphytic growth by sexual species of *Epichloë* causing 'choke' in seed heads and therefore limiting seed yields (Funk *et al.*, 1993, 1994). It is likely that research on and application of *Epichloë* endophytes for turf grasses will increase, as one of the avenues to address reductions and bans on pesticide use (Wiewióra and Żurek, 2023).

Research on endophyte strain variation to enhance the agronomic performance of the host turf cultivar has been limited, possibly due to little need to consider the detrimental impacts on livestock. Selections based on elevated ergovaline alkaloid production of specific endophyte strains, and endophyte-grass associations, have led to endophyte-infected cultivars being developed and deployed for their wildlife deterrent properties (Stewart *et al.*, 2022). Endophyte-infected tall fescue and ryegrass that express a wide range of secondary metabolites have been utilised at airports, sports fields and recreational areas.

While the final use of endophyte-infected turf cultivars does not involve livestock, the production of seed of these cultivars does intersect with livestock production systems. The endophyte strains in these turf grasses produce endophyte alkaloids such as lolitrem and ergovaline that are known to cause endophyte toxicosis in farmed livestock. Risks of toxicity for livestock occur in mixed farming systems such as used in Australia and New Zealand, where livestock may graze seed production stands prior to and/or post-seed harvest. In addition, endophytic straw from the seed harvest may be used as feed for livestock. The toxicity of straw has been of greatest concern in the large seed production systems of the Willamette Valley in Oregon, USA, where large volumes of straw are shipped to Asia for its use in beef production (Craig, 2009). This came to prominence late in the twentieth century when straw burning reduced, and the number of endophyte-infected turf cultivars rose rapidly. In the future, turf breeders may incorporate selected endophyte strains that have no or reduced toxicity to livestock, to enhance pest resistance and possibly other traits, such as the epoxyanthitrem-producing AR37 strain (Stewart, 2005; Popay *et al.*, 2008).

### **13.2 Application for cereals**

Cereal grasses (tribe Hordeae, formerly Triticeae) have for millennia been critical as food for humans and as a feed for livestock (Feuillet and Muehlbauer, 2009). Some wild *Elymus* and *Hordeum* relatives of the domesticated modern cereals harbour a range of *Epichloë* (Card *et al.*, 2014) and at least some are bioactive (Clement *et al.*, 2005). These *Epichloë* have secondary metabolites

that are known for their anti-insect activity in pasture grasses, offering the potential for utilizing them in crops such as barley (*Hordeum vulgare* L.), wheat (*Triticum* spp.) and rye (*Secale cereale* L.) (Simpson *et al.*, 2014; Li *et al.*, 2021; Wang *et al.*, 2022; Popay *et al.*, 2023). Once the endophyte is transferred to a modern cereal, *Epichloë* has proved effective in reducing at least some insect pests and diseases and promoting growth (Hume *et al.*, 2018; Li *et al.*, 2021; Popay *et al.*, 2023).

As with pasture grasses, compatibility with the cereal host genetics has proved critical in the formation of a symbiosis and, in particular, a stable mutualistic *Epichloë* association with the new cereal host. This is not unexpected given the relatively large genetic distance between the original host of the *Epichloë* (wild cereal relative) and the newly inoculated host (domesticated cereal). Where a symbiosis has been established, the most immediate sign of incompatibility is a stunted morphological phenotype of the infected host (Simpson *et al.*, 2014). While this can also occur in pasture grasses (Simpson and Mace, 2012; Simpson *et al.*, 2012), the frequency and magnitude of the responses in phenotype in the cereals have been substantial (Simpson *et al.*, 2014). In outbreeding rye, selection has been possible from the range of expressed phenotypes, while phenotype diversity in wheat has required the use of specific genetics from wheat-alien addition/substitution lines (Johnson *et al.*, 2018).

Given the increasing limitations placed on the use of synthetic chemicals, and the promotion of environmentally friendly agriculture, further development of mammalian-safe *Epichloë* for cereals is likely to contribute to the environmental and sustainability goals of regions such as Europe (Barzman *et al.*, 2015; Lamichhane, 2017) and other areas of the world.

## 14 Genetic manipulation

Finding new chemistry or variation in naturally occurring endophyte strains, to improve on the current suite of selected strains used commercially in ryegrass and tall fescue, is becoming increasingly difficult (Caradus *et al.*, 2021b). Given the increasing breadth of knowledge of the major secondary metabolites and their known bioactivity and gene control, genetic manipulation to form an ideal endophyte for commerce has been investigated. This has opened up new possibilities to design endophyte strains with endophyte secondary metabolite profiles that maximise invertebrate pest protection to the host grass, without harming livestock, a combination of traits that have not been found to date, or do not exist, for strains in nature.

An early example of investigations using genetic manipulation was for the perennial ryegrass Lp1 (AR6) hybrid strain (*E. hybrida*) (Campbell *et al.*, 2017). This was the first selected endophyte strain to be released for use in ryegrass

(branded Endosafe). It had limited success due to concerns over high levels of ergovaline, the ergot alkaloid of most concern for its detrimental effects on livestock (Johnson *et al.*, 2013). Using homologous recombination, gene knock outs were achieved in Lp1 that prevented the production of ergovaline but did not disrupt the compatibility of this strain with the ryegrass host (Panaccione *et al.*, 2001). In a follow-up bioactivity study with modified strains, it was found that the ergot alkaloids were not responsible for the suppression of plant parasitic nematodes (*Pratylenchus scribneri*) (Panaccione *et al.*, 2006b). In addition, the importance of intermediates in the ergot alkaloid pathway was revealed for effects on herbivore responses in a model animal species (Panaccione *et al.*, 2006a).

Application of the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas (CRISPR Associated protein) gene editing system (Shi *et al.*, 2017) to *Epichloë* has been a step change for researchers. This technique has enabled precise gene manipulation of *Epichloë* alkaloids, without off-target effects or introduction of foreign DNA (footprint-less). This newer and safer technology may be more readily accepted by the public, particularly for gene disruption or deletion edits (SDN1 (site-directed nuclease)), leading to faster and more widespread commercial adoption of endophytes developed using CRISPR or other gene-editing techniques (Johnson *et al.*, 2021; Caradus, 2023b).

CRISPR-Cas9 has been successfully used to manipulate ergot alkaloid production in the toxic e19 tall fescue endophyte (*E. coenophiala*) strain. This has overcome the considerable difficulties encountered using older genetic technologies on e19 due to the slow growth of this strain in the laboratory and its triple interspecific hybrid ancestry (Florea *et al.*, 2009; Florea *et al.*, 2021). Florea *et al.* (2021) also successfully applied CRISPR-Cas9 to the ergot alkaloid pathway of the Lp1 ryegrass endophyte. In another perennial ryegrass endophyte, CRISPR-Cas9 has been used to genetically modify the AR37 strain, a strain that had previously been intractable to genetic manipulation (Miller *et al.*, 2022). For AR37, manipulation of the epoxyjanthitrem pathway has enabled its use to further study the bioactivity of pathway intermediates, genes involved in the pathway, and the possibility of revealing bioactivity of other secondary metabolite pathways (Miller *et al.*, 2022).

The use of gene editing in *Epichloë* to improve environmental outcomes, such as reduction in methane emissions, has also been proposed (Caradus, 2023a). An advantage of delivering new traits of value through genetic modification of asexual *Epichloë* is its life cycle which means it can only be maternally inherited and is not transferred to other germplasm through pollen spread (Mirlohi *et al.*, 2004).

## 15 Conclusion

*Epichloë* fungal endophytes, present in widely used temperate pasture grasses such as tall fescue and perennial ryegrass, provide protection against invertebrate pests as well as causing livestock toxicoses. Both the positive and negative effects are due to endophyte-expressed secondary metabolites. Optimising the secondary metabolite profile is key to achieving the desired bioactivity while minimising the unwanted toxicity to livestock. Undoubtedly changes to primary metabolites and the overall functioning of the plant, and interactions with the surrounding biota, are also important in enabling *Epichloë* to impart a range of biotic and abiotic protective properties to host grasses (Hume *et al.*, 2016; Caradus *et al.*, 2021a; Johnson *et al.*, 2021). Beyond pasture grasses, *Epichloë* endophytes have been shown to provide benefits for wildlife deterrence and improving the tolerance of cereals to biotic challenges.

In regions of the world where *Epichloë* is key to the productivity and persistence of the grass host, the trade off in terms of negative impacts on livestock can be addressed in various ways. This includes reducing the intake of mammalian toxins through the use of non-*Epichloë* pasture species, along with changes in pasture and grazing management. In areas where pasture renewal is economically viable, selected strains of endophyte in combination with elite cultivars can be used to replace the previous toxic endophytic pastures. These selected endophytes have proved highly effective, and despite some limitations, they have been rapidly adopted in some markets. There are opportunities to use mammalian safe endophyte strains to extend the use of tall fescue and ryegrass globally without risk of toxicity to livestock.

Enhancing endophytes through genetic manipulation and particularly the new application of targeted gene-editing technology offer new possibilities to elucidate secondary metabolite pathways and their bioactivity and enable science to design endophytes which could benefit livestock farmers and reduce the environmental impacts of livestock farming.

## 16 Future trends

In some regions of the world, *Epichloë* infection is a critical component of the tall fescue and ryegrass pastures that underpin large ruminant livestock production systems. The importance of *Epichloë* in these regions is only likely to grow, as global warming drives climate change, resulting in even greater biotic and abiotic stresses and increased toxicity of old pastures. Given the high reliance on *Epichloë* for pasture performance in these regions, a greater scientific understanding of the grass-endophyte symbiosis could be utilised to enhance *Epichloë*-driven properties in addition to invertebrate pest protection

and drought tolerance. These properties include improving resistance to plant pathogens, synergistic interactions with surrounding biota, and greater tolerance to temperature extremes, flooding, low soil nutrients, salinity, heavy metals and aluminium. In addition, given the effectiveness of *Epichloë* in alleviating environmental stresses for tall fescue and ryegrass, consideration should be given to utilisation of other grass species known to host *Epichloë* to enhance overall pasture supply and resilience of livestock production systems (Caradus and Hume, 2023).

In the regions where *Epichloë* is critical for pastoral production systems, it is likely that research and innovation will continue to investigate and deliver naturally occurring strains that improve the bioprotective properties of grasses while minimising or eliminating the negative impacts on livestock. However, there is a diminishing rate of return from discovery of naturally occurring strains that have novel properties, and due to climate change, there will be a loss of germplasm in the Mediterranean basin, a key centre of diversity for tall fescue and ryegrass and their associated *Epichloë* endophytes. As an avenue to address this, genetic manipulation of endophytes will continue to grow in importance. The CRISPR-Cas9 gene-editing system has already proven to be an effective genetic tool for understanding key endophyte secondary metabolite pathways of *Epichloë*. This technology may drive a new generation of *Epichloë* strains with greater benefits for pastoral-based livestock production systems. Its application to enable *Epichloë* to act as a carrier of new traits to be expressed by the grass-endophyte association for better environmental outcomes will test the extent by which science can understand and manipulate this mutualistic association.

The opportunity to broaden the application of the bioprotective properties of *Epichloë* seen in a particular grass species-endophyte association, by cross species transfer of endophytes to other grasses, is severely hampered by endophyte-grass host incompatibility. While considerable progress has been made in understanding this fundamental aspect of the biology (Johnson *et al.*, 2021), further work is needed to enable the scientific knowledge to deliver problem-solving outcomes.

Beyond the purpose of addressing livestock toxicity, greater global application of mammalian safe selected endophytes will be driven by global climate change, increasing limitations on the use of synthetic chemicals, and the overall need for natural processes to underpin environmentally friendly production of safe food from livestock. Given the rapid pace of change, researchers need to be proactive in understanding the biology of the grass-*Epichloë* association and its effects on the ecosystem thus allowing its effective application in the field.

There are good prospects for the application of *Epichloë* metabolites beyond livestock production systems (Caradus, 2009). These include use as

natural pesticide products and possible use in human medicine. Bulk extracts from some endophyte-infected seed have already been shown to be active in deterring insect pests of lettuce and roses. The application to medicine is still to be pursued but given there are still a number of classes of *Epichloë* secondary metabolites yet to be fully investigated (Johnson *et al.*, 2007; Johnson *et al.*, 2015), the prospects are favourable.

## 17 Where to look for further information

In addition to the numerous references provided throughout this chapter as detailed above, the following will be of value.

For the USA, the following books and websites provide a range of useful online overviews, historic perspective and practical resources on tall fescue-endophyte for pastures and turf:

- Understanding the tall fescue endophyte [https://forages.ca.uky.edu/files/understanding\\_the\\_tall\\_fescue\\_endophyte.pdf](https://forages.ca.uky.edu/files/understanding_the_tall_fescue_endophyte.pdf).
- Tall fescue for the twenty-first century (Fribourg *et al.* 2009).
- The wonder grass: the story of tall fescue in the United States (Ball *et al.*, 2019).
- The Alliance for Grassland Renewal. <https://grasslandrenewal.org>.

For an overview of the importance of endophytes in ryegrass and tall fescue for New Zealand and Australia, along with the development of selected endophytes for these countries and other regions of the world, see the following: Caradus (2023a); Caradus and Johnson (2020); Card *et al.* (2024); Hume and Sewell (2014); Hume *et al.* (2020); Johnson *et al.* (2013); and Reed *et al.* (2011).

For the UK and Europe, see Kauppinen *et al.* (2016); for China, see Song and Nan (2015) and for South America, see Iannone *et al.* (2021).

Since 1990, the international science community have met every 3–4 years to share the latest findings on *Epichloë*. At these symposia, a wide range of topics are presented, ranging from genetics, ecology, mechanisms and trophic interactions, through to the practical application of endophytes for plant protection and production and impacts on livestock. Over recent symposia, the breadth of grass endophytes has been broadened beyond *Epichloë*, as evidence mounts on the critical role of microbes in plant growth. Abstracts from the most recent (10th) Symposium held in Salamanca Spain 2018 can be found here: <https://digital.csic.es/bitstream/10261/167629/1/Book%20of%20abstracts%20ISFEG10%20Salamanca2018.pdf>.

For the taxonomy of *Epichloë*, see Leuchtman *et al.* (2014).

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