

Agronomic advantages conferred by endophyte infection of perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) in Australia

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Abstract. Perennial ryegrass and tall fescue are key grasses of sown pastures in the high-rainfall zone of south-eastern Australia. Ryegrass in naturalised pastures, and in sown seed, is widely infected with *Neotyphodium* fungal endophytes, with toxic endophyte strains occasionally causing toxicosis in livestock. Endophyte infection is also beneficial in sown grasslands, assisting ryegrass hosts to overcome biotic stresses, and tall fescue hosts to overcome biotic and abiotic stresses. We review the literature for Australia and present new data, to examine the agronomic effects of endophyte. Frequency of endophyte infection in old, perennial ryegrass pastures and ecotype-based cultivars is high and, in all pastures, increases with time, providing evidence for endophyte-infected plants having an agronomic advantage over endophyte-free plants. Within a cultivar, agronomic field experiments have compared endophyte-infected with endophyte-free swards. Endophyte significantly improved ryegrass establishment in seven of 19 measurements taken from 12 trials. In mature ryegrass pastures, over half of the experiments found advantages to endophyte infection. Tall fescues infected with a selected endophyte ('AR542') had improved agronomic performance relative to endophyte-free in a majority of experiments, and on occasions, the endophyte was essential for tall fescue persistence. Cultivar × endophyte interactions occurred but were inconsistent. In high-stress environments, endophyte was more important for agronomic performance than difference between cultivars. The relative importance of cultivar and endophyte is discussed, with elite cultivars that are adapted to the region and are infected with elite endophytes being the best avenue to capture the benefits and minimise detrimental endophyte effects on livestock. The major drivers are likely to be insect pests and drought, but evidence is limited.

Additional keywords: dry matter yield, *Festuca arundinacea*, grass population, *Lolium perenne*, *Neotyphodium coenophialum*, *Neotyphodium lolii*.

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Introduction

Perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) are among the most important grass species sown in pastures in the temperate, high-rainfall (>600 mm) zone of south-eastern Australia. Perennial ryegrass occurs in 6 million hectares (Mha) of grasslands (Hill and Donald 1998; Young *et al.* 2013). It is the predominant grass in the cool, temperate, winter–spring rainfall, southern regions, particularly in the state of Victoria where it occurs on 4 Mha. The area of tall fescue is much smaller, the grass being present on 1.1 Mha of sown pastures (Hill and Donald 1998; Young *et al.* 2013). Continental-type tall fescues are particularly suited to the Northern Tablelands and Slopes of New South Wales (NSW), where over half the annual rainfall occurs in summer (Easton *et al.* 1994). In general, these grasses, in combination with subterranean and white clovers (*Trifolium subterraneum* L.

and *T. repens* L., respectively), provide high-quality forage for livestock, and are tolerant of a range of environmental conditions and grazing managements when grown in fertile soils (Easton *et al.* 1994; Reed 1996).

Like several other temperate grasses, perennial ryegrass and tall fescue have co-evolved with the asexual *Neotyphodium* fungal endophyte (Schardl *et al.* 2004), which is now classified as part of the *Epichloë* genus (Leuchtman *et al.* 2014). The grass–endophyte association is asymptomatic and generally regarded as mutualistic, with both the fungus and grass benefiting. The endophyte resides in the apical meristem, colonising new tillers as they are formed, as well as seed. This asexual process is highly efficient, with the endophyte having no other means of proliferation, and it is only maternally inherited in seed (absent in pollen). Seed with viable endophyte is therefore critical for endophyte to affect

pastoral systems based on sown pastures. For the grass host, endophyte infection imparts unique bioactive properties, which increase its tolerance to a range of biotic (e.g. insect predation) and abiotic (e.g. soil water deficit) stresses (Malinowski and Belesky 2000; Popay and Bonos 2005). Although endophyte infection enhances plant performance, some endophyte strains (e.g. 'wild-type' or 'standard' endophyte) cause ill health and productivity losses in grazing livestock (Schmidt and Osborn 1993; Thom *et al.* 2012). To overcome this, 'selected' (sometimes called 'novel'), naturally occurring endophyte strains have been incorporated into elite cultivars and are marketed in USA, New Zealand and Australia (Fletcher 2012; Thom *et al.* 2012; Young *et al.* 2013).

Effects of endophyte in the pastoral livestock systems of Australia are less well studied than in the USA (predominantly tall fescue) and New Zealand (predominantly perennial ryegrass), but are nonetheless significant. Naturalised perennial ryegrass in old pastures of Tasmania, Victoria, South Australia, and NSW is commonly infected at a high frequency with the wild-type strain(s) of endophyte (Reed *et al.* 2000). This animal-toxic endophyte strain(s) causes livestock to suffer a range of disorders, commonly referred to as 'perennial ryegrass toxicosis (PRGT)'. Symptoms include ill health (ryegrass staggers, heat stress), loss of productivity, and mortality. In some years, there are widespread outbreaks of severe perennial ryegrass toxicosis (Reed *et al.* 2005b, 2011). For example, in 2002, almost 100 000 livestock died, mainly sheep, with an estimated similar loss in winter because of the summer–autumn event. These major events are sporadic, with three such epidemics since 1985. Ryegrass staggers in sheep can be observed every year in some regions (Reed *et al.* 2005b), while subclinical losses are also likely in most years and are widespread (Valentine *et al.* 1993b; Foot *et al.* 1994; Lean 2001; Reed *et al.* 2005a). In an economic analysis in 2006, Sackett and Francis (2006) conservatively estimated that perennial ryegrass toxicosis was causing financial losses of AU \$72 million year⁻¹ for Australian sheep and beef producers, with a more recent assessment in 2012 placing this at ~\$100 million year⁻¹ (J. Webb Ware, unpubl. data). The role of endophyte in determining grass productivity and persistence is less clearly defined and has been the subject of conjecture, and farmer understanding has been lacking (Evans 2007). In this paper we review published information and summarise new data on the role of endophyte in plant performance in Australia.

Frequency of endophyte infection—ryegrass

Endophyte infection frequency is a key indicator of the importance of endophyte in the agronomic performance of grass plants in grazed pastures (Hume and Barker 2005). High frequency of endophyte-infected tillers in old pastures and ecotype-based cultivars, and increasing frequency of infection in many pastures over time, all indicate that endophyte-infected plants are better adapted than endophyte-free plants to stresses over the lifetime of the pasture. If there is no advantage to endophyte infection, frequency of infection may decline over time (Leuchtman 1993), due to failure to infect all newly formed tillers, seed, and seedlings, as these are the only avenues for the fungus to maintain the association with the

plant (Gundel *et al.* 2011). Although rates of tiller and seed infection are usually very high, 100% or close, particularly for natural associations (as opposed to artificially inoculated associations), small failures to colonise do occur (Welty *et al.* 1994). This will ultimately result in endophyte frequency in the pasture decreasing rather than being maintained or increasing over time, unless there are selective advantages for endophyte-infected plants.

Studies in Tasmania, Victoria, and NSW report that endophyte is widespread in perennial ryegrass, and that infection frequencies within pastures are high and increase over time. In Tasmania, Guy (1992) reported that all 27 perennial ryegrass pastures sampled from a range of cultivars and sites had endophyte. Within a pasture, frequency of infection averaged 42% (range 4–66%) for <1–5-year-old pastures, and 83% (range 41–94%) for 7–25-year-old pastures. In a further study, initial infection levels of 40–50% increased to ~90% over 1–3 years in four newly sown pastures (Guy and Rowe 2002). Also in Tasmania, Reed *et al.* (2000) cite unpublished work in which a pasture established with 30% endophyte-infected seed had, 5 years later, >80% infection in seedlings recruited from natural reseeding. In south-western Victoria, at two sites with rainfall marginal for perennial ryegrass persistence, two cultivars sown with 79% endophyte-infected seed had 100% infection of surviving plants 4 years later (Cunningham *et al.* 1993). In this region of Victoria, endophyte frequency was 78% at 120 dairy farms in pastures that had a mean age of 22 years (range 0.5–109 years) (Reed *et al.* 2004), while 21 pastures on sheep and beef farms had a mean infection of 85% (Reed *et al.* 2011). In NSW, three pasture surveys for endophyte have been conducted in the Central and Northern Tablelands. Endophyte was present in 75% and 95% of pastures surveyed by Sen (1995) and Wheatley (1997), respectively, while Kahn *et al.* (2003) detected lolitrem B, an endophyte alkaloid of wild-type endophyte-infected ryegrass, in all ryegrass pastures tested. For the infected pastures sampled in the Central Tablelands, 100% of plants were endophyte-infected (Wheatley 2009). In a small-plot experiment at Orange, in the Central Tablelands of NSW, an initial 11% infection in low endophyte treatments increased to 26% after 2.7 years (Wheatley 2009).

The Australian ecotype-derived ryegrass cultivars Victorian and, to a lesser extent, Kangaroo Valley have been the most widely sown perennial ryegrasses in Australia over the last 50+ years, with several uncertified varieties and proprietary cultivars being derived from these (Cunningham *et al.* 1994). Reed *et al.* (2000) proposed that the reputed drought tolerance of Australian ecotypes, particularly Victorian, may be partly due to endophyte infection. A survey of the recognised zones of naturalisation of Victorian and Kangaroo Valley ecotypes in Victoria and NSW, respectively, found all plant populations infected, with an average of 90% endophyte infection within a population (Reed *et al.* 2000). These two varieties occurred in many of the studies listed in the preceding paragraph, and show high rates of endophyte infection in pastures. Similarly in Tasmania, old pastures of Victorian and Kangaroo Valley had 90% infection (Cunningham *et al.* 1994). Seed lots of these varieties are usually infected with endophyte, and at moderate to high frequencies. For Victorian ryegrass, early studies report 63–90% of stored seed lines infected with endophyte (van

Heeswijck and McDonald 1992), while 97% of 58 freshly harvested commercial seed lines in 1991 had viable endophyte, and within infected lines the mean infection frequency was 66% (range 9–85%) (Valentine *et al.* 1993a). In the 1991 study, 71% of Kangaroo Valley seed samples were infected with endophyte, with mean infection of 53% for infected seed lots (range 3–86%). It should be noted that endophyte in seed dies faster than the host grass seed, so length of storage can affect the occurrence of live endophyte in seed lots (Wheatley *et al.* 2007). If this seed is multiplied, endophyte is not transferred, or it occurs at a low frequency in the resulting seed crop.

Plant performance and endophyte—ryegrass

Field experiments measuring performance of perennial ryegrass for endophyte-infected and endophyte-free lines within the same cultivar have been published (Tables 1 and 2) for five regions of south-eastern Australia: the south-east of South Australia, south-western Victoria, South Coast NSW, Central Tablelands NSW, and south-eastern Queensland. In total, we identified publications for 20 experiments utilising nine New Zealand cultivars, predominantly Ellett, and one Australian ecotype, Victorian. Endophyte infection frequencies of the lines were sometimes recorded as ‘high’ and ‘low’, but otherwise they were the measured frequency in the sown seed and/or plots. In an experiment in South Australia, a range of endophyte-infection frequencies was used, and a regression analysis performed to determine effects on agronomic performance (Valentine *et al.* 1993a). Over all experiments, variables measured included seedling weights, population densities of plants and tillers, and dry matter yield of ryegrass.

Establishment

Effects of endophyte on establishment of ryegrass were examined in 13 experiments in two regions, nine in the south-east of South Australia and four in south-western Victoria (Table 1). Of 21 measurements taken, plant number was the main parameter recorded. Relative to endophyte-free ryegrass, responses to endophyte infection ranged from –18 to +72%. Of the measurements statistically analysed, all experiments in Victoria had significant positive responses to endophyte infection (five of 10 measurements) ($P < 0.05$), whereas in South Australia only two of nine experiments had significant positive responses (two of nine measurements) ($P < 0.05$). For the seven statistically significant measurements, responses ranged from +32 to +72% (mean +52%, median +48%) ($P < 0.05$). In the one experiment in Victoria that did not present statistical analyses, positive effects (+30%, +40%) were in the range of significant values reported in other experiments. None of the negative responses to endophyte infection was statistically significant ($P > 0.05$). At Hamilton, Quigley (2000) also recorded interactions with sowing rate. Establishment experiments need to take into account percentage germination of the seed lots being used and potential differences in seedling vigour. Three of the experiments in Victoria report equalising for percentage germination, but at least for the study by Quigley (2000), seed vigour may have differed between lines, as endophyte-free seed

Table 1. Details of 13 published experiments reporting establishment parameters of perennial ryegrass for endophyte-infected compared with endophyte-free seed lines within the same cultivar
* $P < 0.05$; n.s., not significant, $P > 0.05$; n.r., not reported

Location	No. of expts	Cultivar/ecotype	Infection frequency (%)		Measured parameters	Advantage to endophyte-infected	Statistical significance	Reference and notes
			Endophyte-free	Endophyte-infected				
Flaxley and Springton, SE S. Aust.	6	Ellett, Victorian	0	9–87	No. of plants established	–18 to +67%	* 2 of 6 expts (+24, +42%)	Valentine <i>et al.</i> 1993a; ryegrass only
	3	Ellett	0	66, 87	No. of plants established	–5 to +55%	n.s.	Valentine <i>et al.</i> 1993a; with white clover
Hamilton, SW Vic.	1	Ellett	0	80	Plant density at 6 weeks	+51%	*	Reed <i>et al.</i> 1985
		Ariki	0	66	Plant density at 6 weeks	+53%	*	
		Victorian	1	68	Plant density at 6 weeks	+29%	n.s.	
	1	Ellett	0	68	Plant density at 6 weeks	+72%	*	Clark and Reed 1989
		Victorian	1	62	Plant density at 6 weeks	+18%	n.s.	
		Ellett	0	68	Plant weight at 6 weeks	+48%	*	
		Victorian	1	62	Plant weight at 6 weeks	+9%	n.s.	
	1	Ellett	0	75	Plant density at 1 month	+32%	***	Quigley 2000
		Ellett	0	75	Tillers per plant at 4 months	0%	n.s.	
		Ellett	0	75	Plant weight at 4 months	–6%	n.s.	
	1	Ellett, Victorian	0–3	69–77	Plant density at 2 months	+30, +40%	n.r.	Reed 1987

Table 2. Details of 20 published experiments reporting dry matter yields and plant density of perennial ryegrass for endophyte-infected compared with endophyte-free seed lines within the same cultivar

Location	No. of expts	Cultivar/ecotype	Infection frequency (%) Endophyte-free	Infection frequency (%) Endophyte-infected	Measured parameters	Advantage to endophyte-infected	Statistical significance	Reference and notes
Flaxley, SE S. Aust.	1	Ellett	1	88	Total pasture yields years 1 and 2	+7, +5%	*, n.s.	Valentine <i>et al.</i> 1993b
Flaxley and Springton, SE S. Aust.	6	Ellett, Victorian	0	9–87	Yield year 1	No correlation with % endophyte	n.s.	Valentine <i>et al.</i> 1993a; ryegrass only
		Victorian	0	9–85	Yield year 2	No correlation with % endophyte	n.s.	
		Ellett	0	51–87	Yield year 2	+11, +11, +2.5% (*)	* 1 of 3 expts	Signif. correlation with % endophyte
	3	Ellett	0	66, 87	Yield year 1	-11 to +8%	n.s.	Valentine <i>et al.</i> 1993a; with white clover
		Ellett	0	66, 87	Yield year 2	-7 to +20%	* Increase for 2 of 3 expts	
Hamilton, SW Vic.	1	Ellett	0	77	Yield 2.5-year total	-4%	n.r.	Foot <i>et al.</i> 1988
		Victorian	3	69	Yield 2.5-year total	+92%	n.r.	
		Ellett	0	77	Plant density year 3	+67%	n.r.	
		Victorian	3	69	Plant density year 3	+60%	n.r.	
		Ellett	0	68	Plant density year 2	-30%	n.s.	Clark and Reed 1989; Clark <i>et al.</i> 1990
		Victorian	1	62	Plant density year 2	+28%	n.s.	
		Ellett	0	68	Yield year 1	+52%	*	
		Victorian	1	62	Yield year 1	+59%	*	
		Ellett	0	68	Yield year 2	+16%	n.s.	
		Victorian	1	62	Yield year 2	+38%	*	
		Ellett	0	68	Yield 2-year total	+31%	*	
		Victorian	1	62	Yield 2-year total	+46%	*	
	1	Victorian	Low	High	Spring-summer yield year 2	-5%	n.s.	Feely <i>et al.</i> 1994;
		Ellett	0	75	Tiller density at 0.3, 0.75, 1.1, 2.2 years	+25, +37, +32, +74%	*	Quigley 2000
		Ellett	0	75	Total pasture yield for 2.3 years	+10%	* 5 of 8 harvests	
Balmoral and Miminera, SW Vic.	1	Victorian	0–10	78–100	Plant density year 3	+15%	n.r.	Cunningham 1988
		Ellett	0	80–100	Plant density year 3	+75%	n.r.	
		Victorian	0–10	78–100	Yield 3-year total	+12%	n.r.	
		Ellett	0	80–100	Yield 3-year total	+33%	n.r.	
Bega and Nowra, South Coast, New South Wales	2	Yatsyn, Vedette, Ellett	Low	High	Yield year 2	+15 to +69%	* (n.s. for Ellett)	Launders <i>et al.</i> 1996

(continued next page)

Table 2. (continued)

Location	No. of expts	Cultivar/ecotype	Infection frequency (%) Endophyte-free	Infection frequency (%) Endophyte-infected	Measured parameters	Advantage to endophyte-infected	Statistical significance	Reference and notes
Borenore, Central Tablelands, NSW	1	Yatsyn, Vedette, Ellett	Low	High	Ryegrass density year 2	-1 to +130%	* (n.s. for Ellett)	
		Yatsyn, Vedette	0-low	High	Yield year 3	+69, +140%	*	Wheatley 2005a; Bega site only
Orange, Central Tablelands, NSW	1	Lincoln	0	78	Yield 0.6-1.8 years	+14 to +333%	n.s.	Wheatley 2009
		Yatsyn, Nui, Lincoln	6-34	92-97	Yield 0.5-2.5 years (10 harvests)	+2 to +18% (mean 12%)	* 3 of 10 harvests (9, 12, 18%)	Wheatley 2009
Gatton, SE Qld	1	Yatsyn, Nui, Lincoln	6-34	92-97	Plant density years 2 and 3	+2 to +5%	n.s.	
		Bronsyn, Impact, Samson	0-5	79-100	Plant density year 5 Yield 3-year total Plant density year 3	+24% +11 to +18% +186 to +212%	* *	Lowe et al. 2008

was obtained through storage of seed under ambient conditions for 3 years.

Mature pastures

Established ryegrass swards were measured for dry matter (DM) yields and plant or tiller densities in 20 experiments in all states of south-eastern Australia except Tasmania (Table 2). The greatest number of experiments was in the south-east of South Australia (10), where one study measured nine field experiments sown with a range of percentages of endophyte-infected seed. Five experiments were conducted in south-western Victoria (mainly at Hamilton), with the remaining experiments on the South Coast NSW (2), Central Tablelands NSW (2), and south-eastern Queensland (1). Some experiments report only key summary data, whereas others report full seasonal and annual DM yields, botanical composition, and plant densities for 3 years. In Table 2, we present only key data, e.g. Lowe *et al.* (2008). All regions report significant ($P < 0.05$) positive responses to endophyte infection, and of the 18 statistically analysed experiments, 10 report some measurements where endophyte-infected plots had significantly higher yields or greater plant or tiller densities than endophyte-free plots within the same cultivar. The magnitude of statistically significant advantages to endophyte infection ranged from +7% to 212% (mean +44%, median +29%). For two experiments in south-western Victoria, no statistical analyses were published but advantages to endophyte infection were positive and in a range similar to those in other experiments with statistical significance in this region. In some experiments, the agronomic advantage to endophyte-infected lines became statistically significant with increasing time, or significant differences became greater over time. For example, yield advantages for cultivars infected with wild-type endophyte in Queensland were +6%, +31%, and +44% for years 1, 2 and 3, respectively (Lowe *et al.* 2008). In experiments reporting full seasonal data, endophyte effects were greatest in the summer-autumn (Launders *et al.* 1996; Wheatley 2005a; Lowe *et al.* 2008). No experiments report statistically significant ($P < 0.05$) negative responses to endophyte infection.

Cultivar × endophyte interactions

Agronomic performance of endophyte-infected grasses, relative to equivalent endophyte-free grasses, may vary depending on the host grass genetics (Easton 2007). In part, this may be driven by the strong plant genetic control of expression levels of endophyte secondary metabolites, as these compounds affect the degree of insect resistance, and possibly grazing preference (Cosgrove *et al.* 2002) and grazing intensity at low pasture residuals in summer-autumn (Edwards *et al.* 1993) (L. R. Fletcher, R. H. Watson, unpubl. data). Endophyte × cultivar interactions occurred in the experiments we reviewed, but effects were not always consistent. For ryegrass establishment (Table 1), the Victorian ecotype in two experiments at Hamilton was the least responsive of several cultivars to endophyte infection, and these responses were non-significant ($P > 0.05$). However, in a third experiment at Hamilton, the Victorian ecotype had responses similar to,

or possibly greater than, Ellett. In South Australia, there were differing responses for Ellett and Victorian, but this was site-dependent. For DM yields and plant and tiller densities in established pastures (Table 2), two or more cultivars were sown in seven experiments, only five of which had statistical analyses presented. Of these, only two describe cultivar \times endophyte interactions, and these were non-significant ($P > 0.05$) (Lowe *et al.* 2008; Wheatley 2009). On the South Coast of NSW, Vedette was more responsive than Yatsyn to endophyte infection, but only in year 3 (Launders *et al.* 1996; Wheatley 2005a). Of the other experiments (Hamilton and South Australia), cultivar \times endophyte interactions appeared to occur for Ellett and Victorian ryegrasses but these effects were not consistent.

Relative importance of cultivar and endophyte, and selected endophytes

The relative importance of endophyte infection and cultivar in determining grass performance is a critical issue for the plant breeding and seed industry, and for farmers. In New Zealand, Williams *et al.* (2007) concluded: 'the endophyte status of a perennial ryegrass seed line is of crucial importance to its agronomic value, often of greater importance than cultivar identity'. In particular, older New Zealand publications on agronomic performance of new cultivars can now be seen as compromised, as the endophyte status and its agronomic significance was not recognised at that time (Easton 2007). The relative importance of cultivar and endophyte will be determined by the environmental conditions at each site, and these may change between years. At sites or in years with high levels of stress for variables that can be influenced by endophyte infection, grass cultivar will have less influence on agronomic performance, and endophyte infection a greater effect. This is illustrated with the following examples from experiments listed in Table 2.

In the subtropics of south-eastern Queensland, Lowe *et al.* (2008) noted that the enhanced productivity of endophyte-infected compared with endophyte-free lines within the same cultivar (+11 and +18%, depending on endophyte strain) was in the range (10–25%) typically obtained for differences between cultivars at this site over many years of trialling. They advocated that the relative performance of cultivars in agronomic trials should at least consider the endophyte status of the seed sown. A similar situation was evident at Orange, NSW, where mean effects of endophyte infection were +12% for DM yields and the range for three cultivars was 14% (Wheatley 2009). On the South Coast of NSW, where pastures are subject to intense insect pest pressure, the mean difference between cultivars was 13%, whereas the mean impact of endophyte infection was +45% (Launders *et al.* 1996; Wheatley 2005a). In south-western Victoria, two studies report endophyte effects being greater than cultivar effects (Cunningham 1988; Clark and Reed 1989). Experiments have highlighted the value of germplasm from Algeria for its drought tolerance, as gained through summer dormancy (Reed *et al.* 1987; Reed 1996). We hypothesise that endophyte will also be important in this class of perennial ryegrass, but this is yet to be tested.

Selected endophytes

In New Zealand, plant breeders have not considered endophyte *v.* cultivar to be an issue of choice; they have viewed them as complementary, capturing the positive attributes of both (Easton 2007). Shortly after the discovery in the early 1980s that endophyte was a critical determinant of ryegrass performance, the seed industry in New Zealand responded rapidly, reliably producing cultivars with seed that had high levels of viable endophyte. This was the wild-type strain(s) of endophyte, and while this strain maximised ryegrass productivity and persistence, livestock health and productivity were penalised. This situation changed with the discovery of considerable metabolite diversity in naturally occurring endophytes, which lead to the development of 'selected' endophytes. These endophytes deliver improved grass performance over endophyte-free, and they have grass productivity and performance close to, or better than, wild-type endophyte, and no, or greatly reduced, toxicity to livestock (Fletcher 2012). Selected endophytes have been incorporated into elite cultivars, capturing the best plant and fungal genetics to achieve high-yielding ryegrasses of high feed value for livestock. While the elusive 'perfect' endophyte is still to be found (Fletcher 2012), market uptake of this technology has been rapid (Milne 2007).

In contrast to the New Zealand experience, market uptake of selected endophytes in Australia has been slow, due in part to limited agronomic and animal evaluations (Evans 2007). In a recent experiment under dairying in Gippsland, Victoria, ryegrass pastures infected with wild-type and selected (AR1 and AR37) endophytes proved equally productive over a 3-year period (Moate *et al.* 2012). In the subtropics of south-eastern Queensland, both AR1 and wild-type endophytes enhanced ryegrass performance over endophyte-free, but the wild-type was more effective than AR1 (Lowe *et al.* 2008). Endophyte AR1 may also be less effective than wild-type endophyte under the insect pressures of the South Coast NSW, particularly under African black beetle (*Heteronychus arator* F.) attack, but similar to wild-type in other areas of Victoria and NSW (Wheatley 2005a). In New Zealand, variability in agronomic performance between endophyte strains is closely associated with the insect pests against which each strain provides protection (Popay and Thom 2009). Similar effects are being seen in Australia. At Ballarat, in south-western Victoria, an agronomic experiment with eight endophytes in cv. Samson showed significant differences for growth and tiller density in autumn ($P < 0.05$). Relative to endophyte-free, yields in autumn were +17% with AR1, +37% with wild-type, and +61% with AR37, which corresponded with greater numbers of root aphids (*Aploneura lentisci* Pass.) per m² in endophyte-free (31 340) and AR1 (38 930), intermediate numbers in wild-type (17 090), and low numbers in AR37 (4720) (A. J. Popay, J. C. Sewell, D. E. Hume, unpubl. data).

Driving forces for endophyte effects

Endophyte infection can mitigate various abiotic and biotic stresses (Malinowski and Belesky 2000; Popay and Bonos 2005). Abiotic stresses include mineral deficiencies and soil water deficit. Biotic stresses include viruses, nematodes, and

plant diseases, with most work showing effects on a wide range of insect pests. Endophytes may also affect grazing intensity (Edwards *et al.* 1993). In the field, plants are subject to a range of stresses, and when these occur simultaneously, plant productivity and persistence will be greatly diminished. In New Zealand, the agronomic impact of endophyte has largely been attributed to reductions in insect damage, but when combined with soil moisture deficit, effects on plant persistence can be substantial (Popay and Thom 2009). There has been limited work to establish the factors or combination of factors important in determining endophyte effects in Australian pastures.

Summer–autumn soil moisture deficits are common in south-eastern Australia, particularly in the southern winter–spring rainfall zone, and in some years can result in severe droughts. Several Australian field studies allude to the importance of soil water deficits in determining endophyte effects. In Tasmania, two studies report large increases in infection frequency in pastures after 2 years of severe drought (Reed *et al.* 2000) or an unseasonably dry spring–autumn period (Guy and Rowe 2002). In this latter study, the authors believed that this increase did not relate to incidence of significant insect damage. Endophyte effects are reported to be important at sites in south-western Victoria that have marginal rainfall for perennial ryegrass persistence (Cunningham 1988). Irrigated and dryland experiments at Flaxley, South Australia, however, provide no consistent evidence to support this (Valentine *et al.* 1993a). Evidence from controlled studies indicates that endophyte-enhanced drought tolerance effects may be small and inconsistent in perennial ryegrass (Easton 2007), which contrasts with more substantial evidence for this effect in tall fescue (Malinowski and Belesky 2000).

Experimental evidence and anecdotes indicate that endophyte-enhanced agronomic performance in Australia is in part due to increased protection from insect pests. From indoor experiments, McDonald *et al.* (1993) identified that four out of seven insects tested were sensitive to endophyte infection at their early stages of development. Root aphid populations were reduced in the field by endophyte at Ballarat and Gippsland, with variation between endophyte strains also evident (Moate *et al.* 2012) (see above, A. J. Popay, J. C. Sewell, D. E. Hume, unpubl. data). Endophyte also greatly reduced damage by African black beetle on the South Coast of NSW (Wheatley 2005a). In Tasmania, for sites sown with the same batches of seed, the highest infection frequencies occurred in pastures that had suffered damaging insect attacks at least once in their 5-year history (Guy 1992). Endophyte, or endophyte strain, appears to have no effect on redheaded cockchafer (*Adoryphorus couloni*) and blackheaded cockchafer (*Aphodius tasmaniae*) (Watson 2007; Moate *et al.* 2012), insects that are major pests of pasture in south-eastern Australia. Endophyte also has no effect on cereal rust mite (*Abacarus hystrix* Nalepa), a vector for ryegrass mosaic potyvirus (Frost 1993). Differences between endophyte strains have been recorded for some insect pests. In dairy-grazed pastures, AR37-infected ryegrass had lower populations of root aphids, mealybugs (*Pseudococcidae*), and pasture tunnel moths (*Philobota* spp.) than wild-type- or AR1-infected ryegrass (Moate *et al.* 2012). More detailed studies of

pests in Australian pastures and their response to endophyte is needed before conclusions can be made about the reasons for the beneficial effects of endophyte in these environments.

Effects of endophyte on other biotic factors have been tested or observed. At Orange, infection of ryegrass by a leaf spot fungus (*Pyrenophora semeniperda*) was greater on endophyte-free than endophyte-infected treatments for three cultivars (Wheatley 2009). At Gatton, Queensland, the effect of endophyte on crown rust (*Puccinia coronata*) infection was inconsistent (Lowe *et al.* 2008). Endophyte appears to have no effects on viral diseases of ryegrass (Kimbeng and Tasneem 1999). A phytotonic effect of endophyte has been suggested, as endophyte enhanced plant establishment in Victoria in the absence of insects or nematodes (Reed *et al.* 1985).

Endophyte and tall fescue

In contrast to perennial ryegrass, tall fescue sold for pastures in Australia has largely been free of endophyte (*N. coenophialum*) (Easton *et al.* 1994; Reed *et al.* 2005b), although this has changed in recent years with the development of selected endophytes. Endophyte-infected tall fescue can be found naturalised in Australia. Tall fescue on roadsides and riverbanks in Tasmania is infected with endophyte at high frequencies (78–100%) (Guy and Davis 2002), while small areas of grazed pastures in South Australia, Victoria, and NSW have naturalised tall fescue that is infected with endophytes that are toxic to livestock (Pulsford 1950; Wheatley 2005b) (G. C. M. Latch, unpubl. data). In the past, some pastures in northern NSW were sown with imported seed of USA-bred cultivars infected with wild-type endophyte, and these can still be detected (Kahn *et al.* 2003; Harris *et al.* 2008). Since the early 2000s, modern cultivars of endophyte-infected tall fescue have been marketed in Australia. These contain selected endophytes that are non-toxic to sheep and cattle (Parish *et al.* 2003; Hopkins *et al.* 2010), but are not recommended for horses grazing endophyte-infected Mediterranean-type tall fescues (Bourke *et al.* 2009). The use of selected endophytes in Australia stemmed from successful testing and release in the USA (Bouton *et al.* 2002).

The premise for the release of selected endophytes in Australia, and New Zealand, was that endophyte would enhance agronomic performance compared with the same cultivar free of endophyte, and broaden the adapted range (Young *et al.* 2013). This was first tested in small-plot agronomic experiments sown in 2000 at three sites in NSW and Queensland, which showed favourable results within the first year (Wheatley *et al.* 2003). We continued these experiments and commissioned further experiments, with other researchers also independently evaluating these endophyte-infected tall fescues (Harris *et al.* 2008; Reed *et al.* 2008; Boschma *et al.* 2009). In total, 34 experiments were sown from 2000 to 2010 in south-eastern Queensland at Gatton (2); in NSW: South Coast, Hunter Valley, the Slopes, Northern, Central, and Southern Tablelands (17); in Victoria: south-west, central, and northern (12); and in Tasmania (3). Cultivars included the Continental-type tall fescues Jesup, Quantum, and Advance, and the Mediterranean-type tall fescues Resolute and Flecha. Not all cultivars were in all trials. Trials were commissioned or run

by a range of organisations and measured for 1–5 years. Comparisons were made for the same cultivar with endophyte (strain AR542) and without endophyte for DM yields and plant or tiller population densities, in a similar manner to those for the ryegrass experiments. In most cases (30), data were from replicated experiments and analysed statistically, and our interpretation is based on this statistical analysis.

Endophyte infection was advantageous to agronomic performance, within the same cultivar ($P < 0.05$), for the majority of the experiments (24 of 30) statistically analysed. Only one experiment recorded endophyte-infected plots with poorer performance than the equivalent endophyte-free for three cultivars ($P < 0.05$), while in the same experiment, two cultivars showed only positive effects of endophyte infection ($P < 0.05$) (Harris *et al.* 2008). The range of advantages to endophyte infection varied between experiments, and this is well illustrated in the published experiments. For example, in south-western and central Victoria for cultivars Quantum and Resolute, establishment and summer activity were unaffected by endophyte ($P > 0.05$) (Reed *et al.* 2008). For DM yield, cultivars differed in their response, with greater advantages to endophyte infection (mean +23% occurring at more measurements (five of 15) for Quantum than Resolute (mean +11%, at two of 15 measurements) ($P < 0.05$). Final plant density was 11% higher ($P < 0.05$) in one of the four comparisons. By contrast, effects of endophyte at Manilla on the North-West Slopes of NSW were substantial, with final plant populations +490% and final DM yield +132% for Resolute (Boschma *et al.* 2009).

In our unpublished experiments, a similar range of positive endophyte effects was apparent. Agronomic advantages were typically +8 to +100% (mean +38%, median +30%) ($P < 0.05$); advantages beyond this occurred in experiments where endophyte infection was clearly needed for tall fescue to be persistent and productive. In the extreme case, 1 year after sowing at Scone, NSW, five cultivars infected with endophyte were dense and productive, whereas no plants were alive in any of the equivalent endophyte-free plots and for any of the five other endophyte-free tall fescue cultivars. On average for all of the experiments, positive effects of endophyte infection were similar for both the Mediterranean and Continental tall fescue types. Cultivar interactions were sometimes apparent but were inconsistent and require further investigation. As occurred with perennial ryegrass, cultivars within a tall fescue type (Mediterranean or Continental) were the major determinant of agronomic performance when stresses were low, and endophyte became the major determinant as stresses increased. All regions had experiments with positive effects of endophyte infection.

As with ryegrass, the environmental drivers for endophyte-enhanced growth of tall fescue are not well studied. Effects in some regions are most likely due to endophyte reducing damage by insects, and this was clearly the case on the South Coast of NSW, where plots were subject to severe attack by African black beetle (Wheatley *et al.* 2003). Our own observations indicate that root aphid and mealybug populations are also reduced by endophyte. There is strong evidence from USA-based research that endophyte enhances the drought-tolerance of tall fescue (Malinowski and Belesky 2000). This may have

played a role in the experiments we reviewed, and similar to ryegrass, drought and insect pressures together are likely to have the greatest impact. Research is needed for Australian conditions to clarify the key drivers of endophyte-enhanced performance of tall fescue.

Protocols for agronomic trials

Based on the evidence presented above, the endophyte status of lines should be verified to ensure that all agronomic comparisons for perennial ryegrass and tall fescue are valid, even if they are not aiming to evaluate the effects of endophyte (Thom *et al.* 2012). This requires laboratory testing, as endophyte infection is asymptomatic for both seed and plants. The value of this testing is evident in the following studies. At Orange, Wheatley (2009) found that a seed line supplied as endophyte-free was highly infected with viable endophyte, most probably a packaging error by the supplier. In Queensland, Lowe *et al.* (2008) reported an unexpected loss of viable endophyte in seed for one of eight endophyte-infected lines. In both cases, these discrepancies were only discovered by testing tillers for endophyte presence from newly sown plots and pastures. Although we believe such cases are infrequent, it is clearly necessary to validate agronomic experiments for the frequency of viable endophyte infection pre- and post-sowing. From this testing process, it is also important to confirm that infection levels are high for infected lines (>70%), as agronomic performance can be positively correlated with the frequency of endophyte infection (Valentine *et al.* 1993a; Popay *et al.* 1999). Lines identified as being infected at a moderate rate should also be monitored over time, as endophyte infection frequency may increase. Such increases may explain apparent inconsistencies in agronomic performance over time.

Conclusions

From the evidence presented, *Neotyphodium* fungal endophytes of perennial ryegrass and tall fescue are an essential component of the agronomic performance of these grasses in long-lived pastures of south-eastern Australia. Given its importance, research and plant breeding should at least quantify and monitor the level of viable endophyte infection in seed and plant evaluations. The best outcomes for Australian farmers will be achieved through a combination of elite selected endophytes and elite plant genetics adapted to each region, so that perennial ryegrass endophyte toxicosis is eliminated or greatly reduced, and the endophyte-enhancing effects on grass performance are captured. Similarly for tall fescue, the inclusion of selected endophytes will enhance agronomic performance and extend the adaptive range of this grass. A greater understanding of the main drivers for endophyte effects will assist in the development and deployment of new endophyte–grass associations.

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