Endophyte infected perennial ryegrass and tall fescue for New South Wales?

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Introduction

A number of cultivar/endophyte associations in both perennial ryegrass (*Lolium perenne*) and tall fescue (*Schedonorus phoenix*) are now available for evaluation. An endophyte in one cultivar may produce a different outcome to the same endophyte in another cultivar of the same species. There are also different strains of endophyte. Interactions between a number of factors such as cultivar, insect pressure, management, livestock and environmental all play a vital, but often ill defined role, in the impact of grass/endophyte associations on grazing systems in NSW. These associations may be beneficial or result in animal disorders that must be managed in some way.

Endophyte viability in seed

Neotyphodium endophyte is only spread via seed (Siegal *et al* 1985; Rolston *et al* 1994), so for a new endophyte to impact on grazing systems, it must be viable in the seed when sold and sown. A number of studies have demonstrated that when stored under ambient conditions, seed remains viable for much longer than endophyte. The loss in endophyte viability can be significantly influenced by cultivar, age of seed and the interaction between cultivar and age of seed (Wheatley *et al*, unpublished data). Unless particular precautions and seed management procedures are adhered to, seed stored under ambient conditions in NSW and Victoria can only reliably maintain "wild-type" endophyte viability for 12-18 months. Viability of novel endophytes under similar conditions has not been ascertained. Therefore, only fresh seed should be purchased and carryover seed must be stored below 5°C with low relative humidity to maintain endophyte viability. Endophyte can also remain viable and spread via seed in other ways, such as infected seed in the soil bank, undigested seed in dung and seed in hay.

Perennial ryegrass

Occurrence

Perennial ryegrass is a temperate, introduced grass in Australia and even though it is widely sown for pasture, it has also become naturalized throughout this area. It is recommended for pastures due to its ease of establishment and contribution to higher levels of animal production, but its persistence is less than alternative grasses such as phalaris (*Phalaris aquatica*) or Kikuyu (*Pennisetum clandestinum*). Estimates on the area of established perennial ryegrass vary from more than 6 million ha across Australia (Cunningham et al, 1994) to 4.9 million ha in southeastern Australia, with a modeled potential area of adaption of 14.4 million ha in this latter zone (Hill and Donald, 1998). The latter estimate represents 34% of the modeled potential area.

Endophyte strains or types

Wild-type: Produces the alkaloids lolitrem B, peramine and ergovaline. It is widely distributed in all regions where perennial ryegrass grows. A high percentage of fresh seed sold in Australia is infected with "wild-type" endophyte (Quigley and Reed 1999) and usually these bags are not described as such or may be branded as HE (high endophyte). It is unusual for an infection level to be known or provided. In addition, there have been many examples where the endophyte infection level of seed at retail outlets has not been tested. Variable results in the field have led to questions regarding the levels of viable "wild-type" endophyte in perennial ryegrass cultivars at the time of sale (Wheatley 2005).

Novel: AR1 endophyte (free of lolitrem B and ergovaline) is marketed in a number of cultivars. To be marketed as such, there must be a minimum viable endophyte level of 70% at the time of sale.

AR5 and AR6 (free of lolitrem B). AR6 is marketed in the cultivar Extreme. AR5 was marketed as Endosafe in Greenstone.

NEA2 (very low levels of lolitrem B, low levels of peramine and reduced levels of ergovaline (much lower in Bealey)) will be marketed in the cultivar Bealey in 2006.

Bags containing cultivars infected with novel endophytes are branded accordingly.

Endophyte-free: Where the opportunity exists to purchase a perennial ryegrass cultivar either infected with endophyte or endophyte-free, demand for endophyte-free seed is extremely low. One seed company has developed and retails a number of perennial ryegrass cultivars as low or nil endophyte.

Benefits of endophyte association

The benefits of "wild-type" endophyte infected (E+) plants mainly include greater seedling vigour/establishment, production and persistence. An integral part of this is the insect resistance, largely provided by peramine and ergovaline.

Seedling vigour/establishment: This has been reported in NSW and Vic. trials. As an example, a large scale grazing trial at Borenore, NSW, was sown to "wild-type" E+ and endophyte-free (E-) Grasslands Lincoln at the end of August, 1995. When sheep were introduced to the area in mid March, 1996, the E+ perennial ryegrass component of feed on offer was 30% greater than the E- component (1833 kg/ha DM and 1415 kg/ha DM respectively) (Wheatley, unpublished data).

Production/persistence: Comparisons between E+ and E- show variable results, unless there is pressure from insects, particularly African black beetle (*Heteronychus arator*) and possibly environmental factors, and then there is a significant benefit from E+ plants. On the south coast of NSW, it is common for E- stands to be severely thinned or totally lost within one year if African black beetle is active. Early evidence suggests that under the combination of insect damage and environmental factors, production and persistence from AR1 endophyte is comparable to "wild type" in early years, but is less in subsequent years (H.Kemp, pers comm.). Tables 1 and 2 provide some examples of trial results.

Studies in NSW, Victoria and Tasmania demonstrate a high level of "wild-type" E+ plants in established/old perennial ryegrass stands, leading

Table 1. * Comparison between a number of cultivars 'Wild type' endophyte has been standardised to 100

Trial location	AR1 Yield	AR1 plant density
Maffra (Vic)	100	113
Cobden (Vic)	98	102
Trafalgar (Vic)	102	101
Timboon (Vic)	100	105
Deniliquin (NSW)	99	99
Bowral (NSW)	103	99
Overall	100	103

No major insect problems were evident at any site
* Source: Dr. J. Evans, Heritage Seeds Pty. Ltd.

Table 2. ** Comparisons in Bega (NSW) district Site 2. 2002 (Year 2 of trial) Impact WT ('wild type' endophyte) = 100

Cultivar	Total yield (7 harvests)
Bronsyn WT	90
Bronsyn AR1	89
Impact WT	100
Impact AR1	91
Meridian WT	88
Meridian AR1	99

** Source: H. Kemp, DPI, Bega

Table 2. ** Comparisons in Bega (NSW) district Site 1. 1996 (Year 3 of trial)

Cultivar	Dry Matter (t/ha) (mean of 3 reps)			
	Autumn '96 2 harvests	Winter '96 2 harvests	Spring '96 4 harvests	Summer 96/97 2 harvests
Yatsyn high endo	5.59	5.21	11.57	5.80
Yatsyn nil endo	2.16	2.53	8.74	3.22
Vedette high endo	5.18	4.90	10.89	4.19
Vedette low endo	1.74	2.02	5.65	1.09
LSD (0.05)	1.29	1.05	2.07	0.88

** Source: H. Kemp, DPI, Bega

to the assumption that E- plants have not persisted (and E+ have) and that with time, endophyte will be important in determining the long term persistence and production of ryegrass.

Associated animal disorders

Prior to the sale, and associated publicity, of novel endophytes into Australia in 2004, producers related to perennial ryegrass staggers, but not the range of other animal disorders outlined by Hume and Cosgrove in the previous paper.

Staggers: Is a neuromuscular disorder that usually, but not always, occurs in autumn, after a dry spell, when perennial ryegrass constitutes most of the green feed on offer (Wheatley 1997) and as livestock graze into the crown, they are predisposed to high rates of lolitrem B (Keogh *et al* 1996). The occurrence of staggers is regulated by the concentration of lolitrem B in the ryegrass and the intake of lolitrem B. Even though animals may be grazing a mixed pasture, they may only be consuming perennial ryegrass for a period of time. Three serious epidemics have occurred in the more southern states in 1985/86 (Vic and Tas.), 1992/93 (SW Vic.) and February-April 2002 (Vic, Tas and SA) in the last 20 years, with deaths of 29,109 sheep, 448 cattle, 140 deer and 4 horses during the latter occasion (Reed *et al* 2005). While staggers occurs each year in NSW, it is spasmodic and even though hundreds of stock have been lost on individual properties in some years, stock losses are usually low. When it has occurred, producers have supplementary fed animals (reducing lolitrem B intake) or allowed animals to move onto "safe" pastures at their leisure (Wheatley 1997).

Other animal disorders: Insufficient work has been conducted to ascertain the impact of alkaloids, particularly ergovaline, on animal production. Short term losses in milk production have been reported on 2 occasions in SA and NSW. A grazing trial at Borenore, NSW and another in Vic. found that livestock bodyweights only fell during the period in which staggers was experienced and compensatory gain was rapid. There have been anecdotal reports in NSW and Vic. of nervous behaviour in cattle grazing perennial ryegrass in summer. Reed *et al* (2000) found significant variations in alkaloid concentrations in Vic. between populations of the ecotypes Victorian and Kangaroo Valley. Kahn *et al* (2004) found that if perennial ryegrass constituted a significant portion of feed being consumed on the New England region of NSW, then alkaloid levels had the potential to adversely affect animal production.

Preferential grazing

While NZ research has shown the potential for sheep to exhibit a preference towards E- plants at the expense of "wild-type" E+ plants, Wheatley (2001) found that at Orange, NSW, sheep, cattle and horses exhibited no preference, irrespective of whether they had been grazing E+ stands immediately prior to being introduced to the choice, or excluded from perennial ryegrass for several months prior to being introduced.

Management of seedheads

In coastal areas of NSW, agronomists often recommend perennial ryegrass stands not be grazed while seedheads are present – slash if necessary. This is to provide better quality feed to animals and also avoid any problems associated with the presence of "wild-type" endophyte in reproductive tillers. But this is not the case on the tablelands and slopes, where stands run to seed every year and are grazed with no problem apparent. In fact producers are often advised to manage their stands in this manner as a way of maintaining or increasing plant density from this new seed. Perennial ryegrass dominant stands are also cut for hay and subsequently fed back to livestock with the potential to cause animal disorders.

What circumstances have led to the difference in recommendations between coastal and inland regions? Is there a real problem? Is it associated with endophyte knowledge of advisory officers and producers?

Tall fescue

Occurrence

Tall fescue is a temperate, introduced perennial grass that may be included in a pasture mixture or in a pure sward with legumes. While it is productive and persistent in summer rainfall areas and contributes to higher levels of animal production, its use has often been limited by weak seedling vigour and insect damage, particularly from African black beetle (Wheatley et al, 2003). Hill and Donald (1998) estimate the area of tall fescue in south-eastern Australia at 1.1 million ha, only 6.5% of its modeled potential area of 16.8 million ha.

Table 3. ** Increases with MaxP relative to endophyte-free for each cultivar
Results from Year 1 – to autumn 2001

Cultivar	Bega	Armidale	Gatton
Advance			
- Annual yield	+ 38% *	+ 8%	+ 4%
- Greatest seasonal difference	+ 82% *	+ 22% *	+ 17% *
Quantum			
- Annual yield	+ 56% *	+ 16% *	+ 7% *
- Greatest seasonal difference	+ 117% *	+ 18% *	+ 22% *
Resolute			
- Annual yield	+ 246% *	+ 6%	0%
- Greatest seasonal difference	+ 2200% *	+ 6%	+ 5%

* P = < 0.05 ** **Source:** Wheatley *et al* (2003)

Endophyte strains or types

Wild-type: In Australia, tall fescue cultivars sold for grazing systems do not contain the "wild-type" endophyte found in turf cultivars and present in grazing systems in other parts of the world. However, there are some small areas of tall fescue eg. south coast and New England NSW, which have been established for a long period. They are not readily consumed by livestock if alternative feed is available and contain a "wild-type" endophyte (H. Kemp, Wheatley and Hume unpublished data; Kahn *et al* 2004).

Novel: MaxP (AR542) (marketed in USA as MaxQ) is free of ergovaline, but produces lolines which confer insect resistance, but no toxicity to livestock. It is marketed in the summer active cultivars of Advance, Jesup and Quantum and the summer dormant/winter active cultivars of Resolute and Flecha. To be marketed as such, there must be a minimum viable endophyte level of 65% at the time of sale. Bags containing cultivars infected with MaxP are branded accordingly.

Endophyte-free: All commercial grazing cultivars sold in Australia are endophyte-free, unless they are branded as containing MaxP. Therefore they do not produce the range of animal disorders seen in USA as outlined by Hume and Cosgrove in the previous paper.

Benefits of endophyte association

The main benefits from the inclusion of MaxP include persistence (from resistance to insects and climatic/environmental) and production.

Persistence: Plantings in northern NSW (more favourable environment) suggest that cultivars with MaxP maintain up to 200% as many plants as that cultivar without endophyte at the mid point of their estimated lifespan and demonstrate a much greater ability to persist and produce under harsh conditions, such as heat and moisture stress, low soil fertility and competition from weeds (R. Eccles, pers comm.).

MaxP may enhance an existing cultivar, not turn it into a "super plant". The choice of plant type (summer active or summer dormant) can be more important than the effect of endophyte. In very dry summer environments, the primary choice should be a summer dormant cultivar, and endophyte the secondary choice. (R. Eccles, pers comm.).

Where insects, particularly African black beetle, are present, such as south coast, NSW, the inclusion of MaxP is essential, otherwise plants may not even persist beyond the first summer.

Production: Plantings in northern NSW indicate an advantage to the inclusion of MaxP of approx. 10% and up to 30% under harsher climatic conditions (R. Eccles, pers comm.)

In Table 3, endophyte-free plots at Bega were severely damaged by African black beetle and possibly other scarabs such as Pruinose (*Sericesthis sp.*). At Armidale and Gatton there were insufficient insect populations to cause significant damage. Results from years 2 and 3 from these trials also show production increases in favour of MaxP infection (D. Hume, unpublished data)

Overall summary

Novel endophytes have only been tested in Australia for a relatively short period and even though some

trends are evident, long term data is not available. Decisions related to endophyte infected tall fescue appear to be more certain than that for endophyte infected perennial ryegrass. If a producer wishes to sow an endophyte infected cultivar in their grazing system, then they will have to ascertain the most appropriate endophyte/cultivar association. If they choose to sow an endophyte-free cultivar, then plant persistence maybe an issue.

Perennial ryegrass: Producers must initially decide whether they want to sow an endophyte-free cultivar. If not, then the choice between a "wild-type" endophyte/cultivar association and a novel endophyte/cultivar association becomes more difficult. Insufficient research has been conducted into the novel endophyte/cultivar associations commercially available, nor any animal disorders, other than staggers, associated with "wild-type" endophyte. The main considerations will involve the attitude towards potential animal disorders and potential lack of persistence due to damage from insects and other environmental factors.

If a producer wants to avoid any animal disorders, or animal production losses, then the choice is between a novel endophyte and endophyte-free. If animal disorders/production losses are not of concern, then the choice is mainly between "wild-type" endophyte and a novel endophyte.

If a producer is concerned about lack of persistence, particularly due to damage from insects such as African black beetle, then they must decide whether one of the current novel endophytes will provide sufficient protection from insects, or whether to sow "wild-type" endophyte and manage the pasture to avoid animal disorders/production losses that may occur. If an endophyte-free cultivar will persist in a region, then it is an option.

Tall fescue: The inclusion of MaxP endophyte into grazing systems offers advantages over endophyte-free plants in terms of increased production and persistence, as well as the ability to grow it successfully in areas in which endophyte-free cultivars will not persist. Superior insect resistance and greater tolerance of heat and water stress appear to be the greatest advantages. Most of the MaxP infected tall fescue research and commercial sowings have been in northern NSW and far south coast NSW. Until more research is conducted in other areas of potential, then it is not possible to ascertain whether a producer should sow a cultivar infected with MaxP or another cultivar that is endophyte-free.

Future: Consideration needs to be given to the following factors:

a) Producers will need to re-evaluate endophyte/cultivar associations as they become commercially available. More endophytes are being tested in a number of cultivars. This will be an ongoing process.

b) The formation of a multi-disciplinary research group, with industry involvement, to establish agronomic trials in a range of environments, to evaluate the impact of endophyte on production and persistence. Results must be published.

c) Research to determine whether animal production losses are occurring from the consumption of "wild-type" endophyte.

d) Questions over the viability of "wild-type" endophyte in seed in the Australian marketplace have been highlighted by the introduction of novel endophytes. Cultivars with AR1 or MaxP endophytes, have a stated minimum viable endophyte level and are regularly tested. In some cases, ensuring high viable endophyte is being achieved through moisture proof packaging and cool storage of seed. Some cultivars are only being delivered to the retailer on an "exact quantity ordered" basis to reduce seed movement and storage under less than ideal conditions around the country. These novel endophytes attract a premium and it appears that the industry has processes in place to meet the stated minimum viable endophyte levels.

   The situation for "wild-type" endophyte and endophyte-free, appears to be somewhat different. Currently there are no declared standards or up-to-date endophyte viability tests available for producers. This may not be much of a problem for freshly harvested seed, but is as seed ages when stored under ambient conditions (Wheatley 2005). There is a serious need for industry standards for "wild-type" and endophyte-free cultivars, with appropriate quality control systems and information for purchasers of seed at the point of sale.

e) Endophytes and alkaloid levels have been used as a marketing tool to promote the sale of individual cultivars. This has created greater

confusion in the market place. There is an ongoing need to educate advisory officers and producers, so that endophyte decision making is fully informed.

References

Cunningham, P.J., M.J. Blumenthal, M.W. Anderson, K.S. Prakash, and A. Leonforte. 1994. Perennial ryegrass improvement in Australia. N.Z. J. Agric. Res. 37:295-310.

Hill, M.J., and G.E. Donald. 1998. Determination of benefits from pasture improvement. Final report for Australian Meat Research Corporation. Revised edn. CSIRO. Australia. 251p.

Kahn, L., Harris, C. and Lowien, J. 2004. Determining the presence of endophytes and associated toxic alkaloids in New England pastures. Final report. MLA PIRD project (PIRD 2003/No 7).

Keogh, R.G., Tapper, B.A. and Fletcher, R.H. 1996. Distributions of fungal endophyte *Acremonium loliì* , and the alkaloids lolitrem B and peramine, within perennial ryegrass. N.Z. J. Agric. Res. 39:121-127

Quigley, P.E., and K.F.M. Reed. 1999. Endophyte in perennial ryegrass: effect on host plants and livestock. *In* Agriculture Notes Series No. AG0202. Dept. Natural Resources and Environment, Victoria, Australia.

Reed, K.F.M., Leonforte, A., Cunningham, P.J., Walsh, J.R., Roberts, G., Johnstone, G.R. and Kearney, G. 2000. Incidence of ryegrass endophyte (*Neotyphodium loliì*) and diversity of associated alkaloid concentrations among naturalised populations of perennial ryegrass (*Lolium perenne* L.). Aust. J. Agric. Res. 51:569-578

Reed, K.F.M., Scrivener, C.J., Rainsford, K.A. and Walker, L.V. 2005. *Neotyphodium* research and application in Australia. ch 1.pp 43-54. In: *Neotyphodium* in Cool Season Grasses. C.Roberts, C.West and D.Spiers (eds). Blackwell Publishing, Oxford, UK. 0 (ISBN 0 8138 0189 3)

Rolston, M.P., M.D. Hare, G.C.M. Latch, and M.J. Christensen. 1994. *Acremonium* endophyte viability in seeds and effects of storage. Seeds Symposium; seed development and germination. Agron. Soc. N. Z. Special Publication 9:77-82. Seed Symp. Aug 1991.

Siegel, M.R., G.C.M. Latch, and M.C. Johnson. 1985. *Acremonium* fungal endophytes of tall fescue and perennial ryegrass: significance and control. Plant Dis. 69:179-183.

Wheatley, W.M. (1997). Perennial ryegrass (*Lolium perenne*) staggers in the central tablelands, NSW, Australia: A survey of livestock producers. In: Neotyphodium/Grass Interactions. C.W. Bacon and N.S. Hill (eds). Plenum Press, New York. (ISBN 0-306-45688-5) pp 447-449

Wheatley, W.M. 2001. An association between perennial ryegrass endophyte, a leafspot caused by (*Pyrenophora semeniperda*) and preferential grazing by sheep. In: Proc. 3rd International Conference on Harmful and Beneficial Microorganisms in Grassland. Pastures and Turf. Volker H. Paul and Peter D. Dapprich (eds). ISBN:3-935807-03-1 pp71-75

Wheatley, W.M., D.E. Hume, H.W. Kemp, M.S. Monk, K.F. Lowe, A.J. Popay, D.B. Baird, and B.A. Tapper. 2003. Effects of fungal endophyte on the persistence and productivity of tall fescue at 3 sites in eastern Australia. *In* Proc. 11th Aust. Agron. Conf. (CD ROM – Pastures). Geelong, Australia.

Wheatley, W.M. 2005. Endophytes, Quality Assurance and the Seed Trade in eastern Australia. sect V: ch 17. p 351-360. In: *Neotyphodium* in Cool Season Grasses. C.Roberts, C.West and D.Spiers (eds). Blackwell Publishing, Oxford, UK. (ISBN 0 8138 0189 3)