Endophyte – what is it and its significance in New Zealand pastoral agriculture

D E Hume and G P Cosgrove

Research Agronomists, AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North 5301, New Zealand

This paper describes the biology of endophyte in pastoral agriculture and the important role it plays in New Zealand in influencing pasture and animal productivity. With similar systems of pastoral agriculture in Australia and NZ, and 80% of ryegrass seed sold in Australia having endophyte (Reed *et al.* 2005), many lessons learnt in NZ regarding endophyte are applicable to Australia.

Endophyte – what is it?

Endophyte is a general botanical term meaning *within* ("endo") the *plant* ("phyte"). It is now a commonly used term in pastoral agriculture and is used to describe a fungus that may live within the grass plant. In full, it should be referred to as a "fungal endophyte" as bacteria may also be endophytes. In agriculture, we are familiar with fungi mainly because of the diseases they cause such as rust on grass leaves and root rots in lucerne. However the relationship of these *Neotyphodium* fungal endophytes with grass plants does not cause disease, and in fact there are many benefits for the grass plant. In return, the grass provides the fungus with food, a place to live and allows it to spread throughout the environment via the grass seed. Such symbiotic relationships in pastoral agriculture are not new to us, with the most commonly known one being the relationship between legume plants and rhizobia bacteria that live within root nodules. In this relationship, plants like clover and lucerne provide food to the bacteria, and in return the plant gets much needed N that the bacteria fix from the air. While we have known about the significance of the legume-rhizobia partnership since the 19th century, the importance of the grass-endophyte partnership was only discovered relatively recently. For tall fescue this occurred in the late 1970s in the USA and for ryegrass in the early 1980s in NZ.

The fungal endophytes we deal with in important cultivated temperate grasses in Australia and NZ (ryegrass and tall fescue) are characterised by living only in the shoot of grass plants. They cannot live independently outside the grass or spread from one plant to another by producing spores. Endophyte is transferred via seed and usually each new grass tiller that is formed becomes infected as does the seed during reproductive growth (endophyte in seed is discussed in the following paper). Few if any fungal hyphae enter the roots while no hyphae enter the grass pollen. Presence of endophyte in the plant is not apparent to the naked eye as it lives between the plant cells, and therefore requires examination by a microscope or other laboratory tests.

Different species of endophyte infect different grasses and are characterised by producing various chemical compounds, called alkaloids, only in infected plants (Table 1). Not all endophyte-grass combinations produce the same range of alkaloids, and concentrations of alkaloids vary with season, part of the plant, and with factors such as nitrogen and water. The same endophyte can also produce different amounts of alkaloids when present in different grass cultivars. Lolines and peramine are relatively uniformly distributed throughout the shoot, while lolitrem B and ergovaline are primarily concentrated in the base of the shoot, reproductive stem and seed head. All alkaloids increase markedly from mid-spring, to be at their greatest concentrations in summer-autumn.

Benefits and disadvantages of endophyte

The benefits to the grass plant of hosting endophyte are considerable and in ryegrass and tall fescue this may result in increased herbage and root growth, persistence and higher seed production. These positive benefits of endophyte infection may arise from improved tolerance to insect pests, nematodes and plant diseases, better tolerance of drought stress, improved nutrient uptake/efficiency, increases in photosynthesis and reduced overgrazing by livestock. In NZ and Australia, it

Table 1. Alkaloids produced by ryegrasses and tall fescue infected with wild-type endophytes, and their effects on grazing livestock and insects.

	Alkaloid			
Host Grass species	Lolitrem B (lolitrem group)	Ergovaline (ergot alkaloid group)	Peramine	Lolines
Perennial & some hybrid ryegrasses#	Present	Present	Present	Absent
Annual ryegrass#	Absent	Absent	Only in young seedlings	Low amounts
Tall fescue#	Absent	Present	Present	Present
Effects on				
Livestock	Causes ryegrass staggers	Causes heat stress and production losses, particularly in tall fescue	None	None
Insects	Yes, but minor	Yes, but primarily against black beetle	Yes	Yes, large effects

The endophyte species in perennial and some hybrid ryegrasses is *Neotyphodium lolii*, in annual ryegrasses *Neotyphodium occultans*, and in tall fescue *Neotyphodium coenophalium*.

appears that the most important benefits are the greater resistance to feeding by insects and greater tolerance of the heat and drought stress of summer/autumn.

The main disadvantage for farmers associated with endophyte in pastures is the ill heath of grazing livestock, primarily ryegrass staggers (ryegrass only), heat stress and fescue foot (tall fescue only). While these symptoms are visually apparent, research has shown there are also reductions in liveweight gains, milk production, reproductive performance and immuno-systems, and dags/fly strike are increased in sheep.

Both the resistance to insect feeding and impaired animal health/productivity can be attributed to the four main groups of alkaloids that have been identified to date (Table 1). Endophyte-infected tall fescue is characterised by loline alkaloids that afford the plant increased insect resistance over endophyte-infected ryegrass. While ryegrass staggers does not occur in endophyte-infected tall fescue, a greater range of ergot alkaloids is produced (of which ergovaline is one) resulting in greater animal toxicity. The endophyte that infects annual ryegrass produces fewer alkaloids and at low concentrations. Consequently, no animal health problems or production losses have been identified, and benefits to annual ryegrass plant are limited to protection from insects at the seedling stage only.

The advantages and disadvantages of endophyte has created a dilemma for many NZ farmers, and those in the USA using tall fescue; should endophyte-free ryegrass be sown to 'optimise' animal performance and health but with the risk of lower pasture production and persistence, or should endophyte-infected ryegrass be sown to 'optimise' pasture growth and persistence but with the risk of poorer animal production and health?

Endophyte in NZ

The importance of endophyte in NZ has been determined by (a) the widespread use of ryegrass in pasture seed mixes and its predominance in pastures, (b) the high occurrence of endophyte within ryegrass seeds and plants, and (c) conditions that preferentially favour endophyte-infected plants over endophyte-free plants.

Ryegrass has been used extensively in NZ farming since the mid-1800s. In the mid-1980s, approximately 90-95% of all sown grass seed was ryegrass (perennial, hybrid and annual) and 60-70% was perennial ryegrass. The moist temperate climate, moderate-high soil fertility and intensive grazing management conditions generally favour ryegrass growth and survival. For example, a pasture survey in the late 1980s showed ryegrass (/white clover) pastures played a "significant part" in 60-75% of NZ pastures. Combined with this, NZ-bred ryegrass cultivars have in the past been highly infected with wild-type endophyte, which contrasts with those from Europe that are generally endophyte-free. Old pastures (>20-25 years old) in NZ have a high proportion of plants infected with endophyte (70-97%). When pastures are sown with nil or low endophyte seed, endophyte-infected plants invade or dominate so that the % of infected plants increases rapidly within 1-2 years. The main reason for the dominance of endophyte-infected plants is their greater resistance to several insect pests. Combined with this, at some times of the year, livestock can detect the presence of endophyte and may preferentially graze endophyte-free plants and lightly graze endophyte-infected plants depending on the choice and quantity of feed available (Cosgrove *et al.* 2002; Edwards *et al.* 1993).

Effects of endophyte on pasture production

Agronomic trials have shown that endophyte is important for pasture production, particularly over the summer/autumn. Compared with endophyte-free, endophyte-infected ryegrass may typically have 15 to 30 % higher production over this period, and up to 77% at some harvests. Much of the lower production of endophyte-free pastures can be attributed to the feeding damage by insects such as Argentine stem weevil, African black beetle and pasture mealy bug (Popay *et al.* 1999). The cost of pasture damage caused by Argentine stem weevil was estimated to be NZ$46-$202M per annum (Prestidge *et al.* 1991). Since that analysis was conducted a biocontrol agent has been released to reduce populations of Argentine stem weevil, so current costs of pasture damage due to this pest alone are likely to be considerably lower. However, feeding by other pests does favour endophyte-infected plants and affects pasture productivity of endophyte-free ryegrass and tall fescue.

Endophyte-infected ryegrass pastures are reported to have lower proportions of white clover. This effect appears to be due to not only greater competition from endophyte-infected ryegrass plants but also an allelopathic effect that reduces white clover growth. The consequences of lower clover growth are less N fixation, so eventually less N available for pasture growth, and lower feed quality as less of the sward is composed of high quality legumes.

Effects of endophyte on grazing livestock

The most apparent effect of endophyte in ryegrass is the occurrence of ryegrass staggers during summer/autumn, caused by lolitrem B. However, intensive research trials with sheep have shown that other alkaloids produced by endophyte, such as ergovaline, also affect animal health (increases the prevalence of dags and so the potential for flystrike) and restricts animal growth rates primarily in the summer-autumn period (Table 2). The ergovaline produced by endophyte-infected ryegrass increases body temperature and can induce heat stress in animals. This has been implicated in secondary animal health disorders such as pneumonia in lambs (Black *et al.* 2005). Heat stress is obviously more significant in Australia than New Zealand. Alkaloids in general may also reduce feed intake (Watson *et al.* 1999).

There have been few studies to directly compare the effects of endophyte on sheep and cattle grazing similar pastures under similar environmental conditions. The effects on cattle appear, in general, to be less than on sheep and in several trials not significant. In one study sheep appeared to be more sensitive to increasing dietary concentrations of ergovaline than were beef heifers (Layton *et al.* 2004), however this comparison is tenuous because the sheep were in a different environment to the cattle. Short-term grazing studies measuring milk production in dairy cows, have shown inconsistent effects. However when assessed in a systems trial over 3 years in the Waikato, dairy cows on pastures with wild-type endophyte in ryegrass produced 8% less milk per ha than those on novel AR1 (non-toxic) endophyte pastures (Bluett *et al.* 2003). A similar 3-year systems trial in Northland showed a smaller effect on milk production (Ussher 2003).

Table 2. Effects of endophyte on lambs in summer and autumn (mean of 5 trials over 3 years, Canterbury) (Fletcher et al. 1999)

	Ryegrass Staggers Score (0-5 scale, 0=Nil)	Deaths due to Ryegrass Staggers#	Dags (0-5 scale)	Flystrike	Liveweight gain (g/ha/day)	Rectal temperature (°C)	Respiration rate (breaths/minute)
Wild-type endophyte	3.1 a	5-11%	1.5 a	22 a	40 b	40.6 a	99 a
No endophyte	0.4 b	0 %	0.4 b	2 b	110 a	40.2 b	73 b

Means within a column with the same letter are not significantly different ($P<0.05$)
\# measured from a separate grazing systems trial over 3 years with ewes/lambs (Fletcher 1999)

On a national basis, Prestidge *et al.* (1991) calculated a financial cost of NZ $43M per annum (1991 $) of ryegrass staggers in Canterbury and Hawkes Bay where outbreaks are most common and severe. This was estimated from losses due to reduced liveweights and deaths. This was a conservative estimate based on sheep production systems only, and would be greater if effects on cattle and other regions of NZ were included in the analysis. A more recent analysis has calculated losses due to wild-type endophyte at NZ $200/ha/year in both sheep and dairy systems (L. R. Fletcher unpublished data).

There are few reports of endophyte toxicity from tall fescue in NZ and Australia. Certified seed of pasture cultivars of tall fescue has been sold as free of endophyte, except for some early lines of Demeter. Toxicity is still possible if roadsides and banks of water ways are grazed as these are usually heavily infected with toxic endophyte. In Northland, NZ, some pastures may contain endophyte-infected tall fescue which can present problems for pasture management and animal health.

How to avoid the worst of endophyte

Several options are available to avoid or minimise the negative effects of endophyte on animal health and production.

Using Nil endophyte ryegrass and tall fescue

When the link was made between endophyte and ill-health in livestock, an easy answer was to eliminate the endophyte from sown seed. This was used extensively in the USA but it soon became apparent that this solution was unacceptable in practice, as endophyte-free tall fescue pastures were too short-lived in stressful environments. This option is still available in the USA today, but endophyte-infected tall fescue seed dominates seed sales. While endophyte-free ryegrass is an option for NZ, its use is limited to regions with low climatic stresses and few pests e.g. Southland, West Coast or to farming systems where stress is minimised (e.g. irrigation) or where a short pasture life is "acceptable" (e.g. dairying or cropping).

Alternative pasture species/crops

With most of the detrimental effects of endophyte on animal health and productivity occurring during the warm seasons of the year, farmers can plan to have other sources of non-endophytic feeds available during these times. These may be pastures with non-endophytic grasses (e.g. prairie grass, cocksfoot), legumes (lucerne), herbs (chicory, plantain) and brassicas. These options may be sown in separate paddocks as specialist pastures, or within ryegrass paddocks (e.g. increased white clover content) so as to dilute the intake of endophyte toxins. The scope for achieving these options may be limited in drought prone areas where animals are most at risk, and in dryland hill country where cultivatable land is a small proportion of the total farm. Farmers in NZ however have found that ryegrass staggers can occur on pastures with low (<20%) ryegrass contents in the sward. Conserved feeds (e.g. hay and silage) may also be used but not from endophyte-infected grasses as the toxic alkaloids are still present e.g. use conserved maize.

Grazing management

With the most toxic herbage (i.e. the highest concentrations of lolitrems and ergovaline) being at the base of the grass plant, a quick rotational grazing management (e.g. daily shifts) where animals remove mainly the upper leaf, can be used to minimise toxin intake and reduce or eliminate ryegrass staggers (Keogh and Clements 1993). This may be most effective when ryegrass staggers is a short-term problem, but its effectiveness is limited when pastures are toxic for extended periods and when regrowth of the grass is limited by dry conditions.

Using selected novel endophytes

Dr Garry Latch and his team in NZ investigated the possibility that within an endophyte species there would be sufficient variation to help solve the endophyte dilemma. Could some endophyte strains give the benefits of plant persistence and production (mainly through insect resistance), and yet be non-toxic or safer for grazing livestock than the wild-type endophyte found in most pastures and ryegrass cultivars? Considerable research showed that within each grass species different strains of endophyte existed and produced different alkaloids. With the knowledge of which alkaloids were toxic to grazing animals and which were deterrent or toxic to insect pests (Table 1), novel endophytes have been selected, and have been available in several cultivars of ryegrass in NZ for over a decade now.

The most successful has been the AR1 endophyte strain in ryegrass. Cultivars infected with AR1 offer improved insect tolerance over the same cultivar endophyte-free but do not cause the animal toxicity or reductions in animal performance as found with the wild-type endophyte. AR1 has been rapidly taken up by the farming industry as the best currently available option for dealing with the endophyte dilemma. AR1 was first released in limited amounts in 2001, and over the last 2 years 40-50% of sales of proprietary perennial ryegrasses have been cultivars infected with AR1. In its four years out on farms, AR1 has proved entirely satisfactory if managed appropriately.

Three other selected endophytes are also available in ryegrass (AR5, AR6, NEA2). These provide increased protection from insects such as African black beetle, but at the risk of having some alkaloids that may be detrimental to animal health/ production.

One selected endophyte is available for tall fescue (MaxP). This endophyte strain does not cause the losses in animal production and ill health as does the wild-type endophyte. It does however enhance pasture productivity and persistence compared with the same cultivar free of endophyte.

Further developments are expected as new endophyte strains are developed, and plant-endophyte associations are refined in both ryegrass and tall fescue.

Other options for the future

While considerable effort has gone into selecting endophytes and plants to reduce or eliminate toxicity, selection for resistance to ryegrass staggers in sheep has received limited attention. Morris *et al.* (1999) have shown it is a heritable trait in sheep, but these experimental selection lines are not currently available commercially in NZ. However, with rapid advances in the use of genetic tools in selection programmes, this may become a viable option in the near future.

It could be possible to use feed additives to reduce the metabolic activity of alkaloids. Currently in NZ there is no use of prophylactics or vaccines that have been reported by independent scientific tests to be cost effective in eliminating ryegrass staggers, endophyte toxicoses, or reduced animal performance. The effect of practices that enhance the general nutrition and health of the animal is not known. Fescue toxicosis in foaling mares in USA can be controlled by administering a drug Domperidone.

Summary

In NZ, endophyte plays a major role in maintaining high producing and persistent ryegrass pastures, primarily through the protection it provides to plants against a number of insect pests. However, the wild-type endophyte is widespread and can cause detrimental effects to grazing livestock through the production of toxic alkaloids. The most effective solution to the endophyte dilemma in ryegrass, is the use of selected novel endophytes to manage pasture pests and animal productivity. Industry uptake of this technology has been rapid for the endophyte AR1, with AR1 now becoming an industry standard. A novel endophyte in tall fescue is also likely to increase the use of this grass species as it is animal safe and improves grass production and persistence. Further novel endophyte developments are likely to present even more options for the farmer in the future.

References

Black, H., Alley, M.R., and Goodwin-Ray, KA. (2005) Heat stress as a manageable risk factor to mitigate pneumonia in lambs. New Zealand Veterinary Journal, 53, 91-92.

Bluett, S.J., Thom, E.R., Clark, D.A., MacDonald, K.A. and Minnee, E.M.K. (2003) Milksolids production from cows grazing perennial ryegrass containing AR1 or wild endophyte. Proceedings of the New Zealand Grassland Association, 65, 83-90.

Cosgrove, G.P., Anderson, C.B., Phillot, M., Nyfeler, D., Hume, D.E., Parsons, A.J. and Lane, G.A. (2002) The effects of endophyte alkaloids on diet selection by sheep. Proceedings of the New Zealand Society of Animal Production, 62, 167-170.

Edwards, G.R., Lucas, R.J., and Johnson, M.R. (1993) Grazing preference for pasture species by sheep is affected by endophyte and nitrogen fertility. Proceedings of the New Zealand Grassland Association, 55, 137-141.

Fletcher, LR. (1999) "Non-toxic" endophytes in ryegrass and their effect on livestock health and production. pp. 133-139. *In*: Ryegrass Endophyte - An Essential New Zealand Symbiosis. Grassland Research and Practice Series No. 7. Eds. Woodfield, D.R. and Matthew, C. New Zealand Grassland Association, Palmerston North.

Keogh, RG. and Clements, R.J. (1993) Grazing management: A basis for control of ryegrass staggers. *In*: Proceedings of the Second International Symposium on Acremonium/ Grass Interactions" pp. 129-131 Eds. Hume, D.E., Latch, G.C.M. and Easton, H.S., AgResearch.

Layton, D.L., Fletcher, L.R., Litherland, A.J., Scannell, M.G., Sprosen, J., Hoogendoorn, C.J. and Lambert, M.G. (2004) Effect of ergot alkaloids on liveweight gain and urine lysergol level in ewe hoggets and heifers. Proceedings of the New Zealand Society of Animal Production, 64, 192-196.

Morris, C.A., Towers, N.R. and Amyes, N.C. (1999) Six years of selection responses for resistance or susceptibility to ryegrass staggers in sheep. pp. 27-31. *In:* Ryegrass Endophyte - An Essential New Zealand Symbiosis. Grassland Research and Practice Series No. 7. Eds. Woodfield, D.R. and Matthew, C. New Zealand Grassland Association, Palmerston North.

Popay, A.J., Hume, D.E., Baltus, J.G., Latch, G.C.M., Tapper, B.A., Lyons, T.B., Cooper, B.M., Pennell, C., Eerens, J.P.J. and Marshall, S.L. (1999) Field performance of perennial ryegrass (*Lolium perenne*) infected with toxin-free fungal endophytes (*Neotyphodium* spp.). pp. 113-122. *In:* Ryegrass Endophyte - An Essential New Zealand Symbiosis. Grassland Research and Practice Series No. 7. Eds. Woodfield, D.R. and Matthew, C. New Zealand Grassland Association, Palmerston North.

Prestidge, R.A., Barker, G.M. and Pottinger, R.P. (1991) The economic cost of Argentine stem weevil in pastures in New Zealand. Proceedings of the New Zealand Weed and Pest Control Conference, 44, 165-170.

Reed, K., Scrivener, C.J., Rainsford, K.A. and Walker, L.V. (2005) *Neotyphodium* research and application in Australia. pp. 43-54. *In: Neotyphodium* in Cool-Season Grasses. Eds. C. A. Roberts, C.A., West, C.P. and Spiers, D.E. Blackwell Publishing, Ames, Iowa, USA.

Ussher, G. (2003) Northlands pasture toxin project. pp. 62-64. *In:* New Zealand Large Herds Association. Eds. New Zealand Large Herds Association Inc., Paihia.

Watson, R.H., Keogh, R.G. and McDonald, M.F. (1999) Ewe reproductive performance and growth rate of suckling-lambs on endophyte-infected perennial ryegrass pasture. pp. 19-26. *In:* Ryegrass Endophyte - An Essential New Zealand Symbiosis. Grassland Research and Practice Series No. 7. Eds. Woodfield, D.R. and Matthew, C. New Zealand Grassland Association, Palmerston North.

Recommended reading

Proceedings of the Second International Symposium on Acremonium/Grass Interactions: Plenary Papers 1993 (Eds. Hume, D.E., Latch, G.C.M. and Easton, H.S.). AgResearch, Palmerston North.

Ryegrass Endophyte - An Essential New Zealand Symbiosis. Grassland Research and Practice Series No. 7. 1999 (Eds. Woodfield, D.R. and Matthew, C.). New Zealand Grassland Association, Palmerston North.

Neotyphodium in Cool-Season Grasses. 2005 (Eds. Roberts, C.A., West, C.P. and Spiers, D.E.). Blackwell Publishing, Ames, IA.

Perennial Ryegrass Toxicosis. 2005 (Ed. Reed, K.F.M.). DPI, Victoria.