

Ryegrass Endophyte: An Up-to-Date Review of its Effects

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1.0 POPULAR SUMMARY

- Most ryegrass based pastures grazed by Merinos will contain endophyte.
- Endophytes are fungi that exist inside plants and are transferred via seed.
- Endophyte exists in a normal symbiotic relationship with ryegrass protecting it from insect attack and offering other advantages.
- Endophytes produce a wide range of compounds including peramine, lolitrem B and ergovaline.
- Peramine offers some protection to the plant from insect attack, especially argentine stem weevil.
- Lolitrem B is a neurotoxin causing ryegrass staggers in animals.
- Ergovaline lowers blood prolactin levels and causes heat stress.
- Some of the above compounds, in combination with other unidentified compounds, depress intake, liveweight gain and cause scouring and dags in sheep.
- Non-toxic strains of endophyte have been selected.
- The most promising is AR1 which produces peramine and protects the plant from insect attack with no ill effects on grazing animals. It is available commercially.
- A number of prevention and control measures against the toxins have been tried, but the only one offering complete protection is to replace the resident ryegrass pastures with AR1 ryegrass.
- Establishment of AR1 requires a spray/crop or double spray treatment to reduce toxic seed.
- Ingress of wild type ryegrass can occur after three or four years and precautions should be taken to prevent seed entry.

2.0 REVIEW: ENDOPHYTES IN NZ PASTURES

2.1 Introduction

Perennial ryegrasses (PRG) is the most widely sown species in NZ pastures and has been intensively researched since the 1920s (Hunt & Easton, 1989). However, as late as 1980, the discoveries that the endophytic fungus, *Neotyphodium lolii*, caused ryegrass staggers (Fletcher & Harvey, 1981) and protected the plant from Argentine stem weevil (ASW) (Prestidge *et al.*, 1982) have required a total re-examination of all we thought we knew about ryegrass pastures. Endophyte research has also enabled us to revise our understanding of tall fescue.

The endophytic fungi in PRG and tall fescue had been described in 1933 (Sampson, 1933) and further studied in the 1940s by NZ scientists (Neill, 1941), and the possibility that they caused livestock disorders was raised and investigated (Cunningham, 1958). However, the experiments failed to make the suggested link, and another 20 years passed before the significance of the endophytes was established.

There was a considerable amount of serendipity about the crucial initial discoveries (Easton *et al.*, 2001). Since then, endophyte research has been focused and multi-disciplinary. Another feature of the work has been the importance of international contacts, and in particular the close and very useful interchange with research groups in the south east of the USA (Easton *et al.*, 2001).

When the endophyte of PRG was first discovered it was named *Acremonium lolii*, however it was later reclassified as *Neotyphodium lolii* (Fletcher & Easton pers. comm.) on the basis of the alkaloids produced (Glenn & Bacon, 1997).

2.2 Endophyte in New Zealand Pastures

Perennial ryegrass (*Lolium perenne*) is naturally infected with the endophyte, *Neotyphodium lolii*, but other endophytic fungi are also regularly found in perennial ryegrass (Latch *et al.*, 1984). The influence of the latter on the pasture-livestock system is unknown and probably small. They are not considered in this discussion, and the word "endophyte" refers here to *N. lolii* or related species in other grass hosts.

Ryegrass collected from old pastures throughout NZ has regularly been found to be infected with *N. lolii* (Prestidge *et al.*, 1985; Wedderburn *et al.*, 1989; Widdup & Ryan, 1992), as has ryegrass collected in Europe (Lewis *et al.*, 1997). Endophyte was first noted in ryegrass in NZ 60 years ago (Neill, 1940). Other grass genera and species are similarly infected with related endophytes, notably tall fescue (*Festuca arundinacea*) (Siegel *et al.*, 1987).

New Zealand experience is that perennial ryegrass in most old pastures is normally infected at very high frequency. However, in the south of NZ, infection rates are lower, and some old pastures are nearly free of endophyte (Widdup & Ryan, 1992). Of 50 populations examined, two were less than 20% infected, but 29 populations were more than 60% infected. Naturally occurring hybrid ryegrass may also be infected with the perennial ryegrass endophyte (Piggot *et al.*, 1988).

New Zealand pastures have higher levels of infection than those studied overseas. Of 54 populations collected in north-west Spain, 15 were free of endophyte (Oliveira & Castro, 1998), and only 12 populations were 60% or more infected. Similar results were observed with French populations (Ravel *et al.*, 1994). These infection levels are lower than those observed in southern NZ (Widdup & Ryan, 1992).

2.3 The Effects of Endophyte on Various Aspects of Pasture Yield & Production

Endophyte is essential to the thrift of perennial ryegrass pastures in NZ because it protects ryegrass from invertebrate pests, notably Argentine stem weevil (ASW) (*Listronotus bonariensis*) (Mortimer & di Menna, 1983; Prestidge & Ball, 1993; Prestidge *et al.*, 1982). Adult ASW are less likely to feed on infected ryegrass (Barker *et al.*, 1984d; Rowan & Gaynor, 1986). Less eggs and larvae are present in infected swards (Gaynor & Hunt, 1983), those present survive less well (Barker *et al.*, 1984c) and larvae attacking ryegrass stems bore less vigorously into infected plants (Prestidge & Gallagher, 1988). In Manawatu, 70% of tillers were observed to be damaged in late summer in endophyte-free swards but negligible damage was observed in infected swards (Popay & Mainland, 1991). Under pressure from ASW, the frequency of endophyte infection increases rapidly as non-infected plants die and are replaced by expansion of infected plants (Hume & Brock, 1997; Prestidge *et al.*, 1984).

ASW has been shown to be an important factor in pasture failure in Canterbury (Goldson, 1982) and much of the North Island (Barker *et al.*, 1984b). Yield responses of 50-60% in mixed ryegrass-white clover swards were achieved by protecting from ASW with insecticide (Kain *et al.*, 1982). In 1991, pasture damage by ASW was estimated as costing NZ \$46-\$200m annually (Prestidge *et al.*, 1991). Before endophyte was shown to provide protection, it had been noted that different seed lines of perennial ryegrass differed in their vulnerability to attack (Kain *et al.*, 1982), and this was thought to indicate genetic or varietal differences.

Endophyte also protects perennial ryegrass from other invertebrates (Prestidge & Ball, 1993). Black beetle (*Heteronychus arator*) is intermittently a serious pest in northern NZ (East *et al.*, 1981). Endophyte-free ryegrass can be quickly destroyed by black beetle (Lee *et al.*, 1982), but endophyte deters feeding by the adults, and egg and larval numbers are reduced (Ball & Prestidge, 1992). Various nematode species, some endo-parasitic, others free-living in the root zone, are found in ryegrass pastures, and there is some evidence that populations are affected by endophyte infection (Eerens *et al.*, 1998b; Stewart *et al.*, 1993). There is very little NZ information on the importance of grass-associated nematodes in pastures (Watson *et al.*, 1986; Yeates & Prestidge, 1986). Pasture mealy bug (*Balanococcus poae*) is an insect frequently present in pastures but of unknown importance. It is sensitive to the presence of endophyte (Pearson, 1989; Pennell & Ball, 1999). Mealy bug presence is trivial in pastures intensively infected with

endophyte, but large numbers may build up in endophyte-free pastures in late summer in dryland conditions (C.G. Pennell, unpub. cited by Easton, 1999). Serious infestation is associated with poor ryegrass growth and persistence, but cause and effect are difficult to establish. Cutworm (*Graphania mutans*) is another insect that is sensitive to endophyte (Dymock *et al.*, 1989b). It is found throughout NZ but is of unknown significance.

Evidence that ryegrass shows greater growth (Latch *et al.*, 1985) or stress tolerance when infected with endophyte, as has been widely reported for tall fescue (West & Gwinn, 1993), has proved to be inconsistent. Intensive growth cabinet and glasshouse-based experiments of a few weeks duration have not shown any endophyte effects on growth or stress tolerance (Barker *et al.*, 1997; Eerens *et al.*, 1998c; Hume *et al.*, 1993; Easton & Rolston, unpub. data cited by Easton 1999; Easton & Hume, unpub. data cited by Easton, 1999). Field experiments are difficult to protect from all invertebrate pressure factors, but a series of row trials run over 2-4 years, where no serious invertebrate pressure was observed, has indicated significant advantage to ryegrass naturally infected with endophyte or infected with certain strains (Hume & Latch, unpub. data cited by Easton 1999). Other field experiments have indicated no such effects (Eerens *et al.*, 1998a; Widdup & Ryan, 1992). Likewise, results in France were inconsistent, but endophyte conferred some advantage to ryegrass, in the absence of apparent invertebrate attack, in plots at more stressful sites (Ravel *et al.*, 1995).

Ryegrass infected with endophyte may be less severely grazed by livestock than uninfected swards (Edwards *et al.*, 1993). Sheep offered a choice, grazed uninfected swards closer to the ground, and further into the leaf sheath horizon, especially after the first few days. Similar differences have been documented in a non-choice grazing experiment by R.H. Watson (cited by Easton, 1999). If such differences in grazing pressure were sustained over time, they could, in the absence of any other factors, lead to a more rapid decline in the endophyte-free sward.

Whether due to pest pressure, drought tolerance or any other mechanism, endophyte-infected ryegrass pastures produce more dry matter than endophyte-free pastures. In a recent series of trials at six sites throughout NZ (Popay *et al.*, 1999), endophyte-infected ryegrass consistently out-yielded endophyte-free ryegrass. Averaged over three years and the six sites, the difference was more than 20% in summer, and more than 30% in autumn. At some sites and in some years, the difference was much greater than this.

To the list of adverse effects can be added a trend for poorer clover growth in endophyte-infected pasture (Stevens & Hickey, 1990; Sutherland & Hoglund, 1989; Valentine *et al.*, 1993). The effect is not always observed (Eerens *et al.*, 1998a), and is mostly an inverse effect of ryegrass vigour (Prestidge *et al.*, 1992). Direct inhibitor (allelopathic) effects on white clover have been documented (Sutherland *et al.*, 1999), but apparent stimulatory effects have also been noted (Eerens *et al.*, 1998d). Livestock may preferentially graze clover in a mixed sward (Milne *et al.*, 1982). This preference is partial (Parsons *et al.*, 1994), and more active avoidance of ryegrass if infected may intensify it. Alternatively, if clover is growing evenly through a grass sward, rather than in a mosaic, endophyte by discouraging close grazing by stock may protect the clover stolons. Poorer clover content in a ryegrass pasture will exacerbate any negative effects of endophyte on livestock performance, and these two factors have been confounded in some experiments.

However, ryegrass endophyte contributes to livestock productivity, by ensuring a sustained leafy ryegrass pasture in stressful situations. The alternative to endophyte-infected ryegrass in many situations would be bare ground, or pasture dominated by *Poa* spp or tropical grasses of poor quality (Campbell *et al.*, 1996). Endophyte-infected ryegrass pastures generally provide more digestible dry matter to grazing livestock than endophyte-free pastures.

The discovery of the novel AR1 endophyte could completely change this scenario as outlined later (Easton *et al.*, 2001).

2.4 Alkaloid Metabolites Produced by Endophyte

Before 1980, it was thought likely that a fungus was involved with ryegrass staggers, and research aimed to isolate the chemical compound(s) responsible. The discovery of the role of the endophyte hastened this process, and lolitrem B was isolated and described (Gallagher *et al.*, 1981). Several related compounds have been identified, but lolitrem B is the most abundant and perhaps the most potent (Miles *et al.*, 1994). Paxilline is similar to lolitrem B and is a tremorgen itself (McLeay & Smith, 1999; Fletcher *et al.*, 1993), but much milder (Easton pers. comm.). Mild staggers has occurred in the absence of lolitrem B (Fletcher *et al.*, 1993) and this may have been due to paxilline or other unidentified neurotoxins. The main compound responsible for protection from ASW was shown to be different, and peramine was isolated and described (Rowan & Gaynor, 1986). USA research had identified ergovaline as the primary fescue toxin, and this compound was later found in endophyte-infected PRG (Rowan & Shaw 1987). Ergovaline and lolitrem B, primarily studied for their effects on livestock, also offer protection against invertebrate pests (Dymock *et al.*, 1989; Ball *et al.*, 1997). Co-evolution of endophyte and host is reflected in the array of compounds occurring in different endophyte-infected grasses (Lane *et al.*, 2000).

Endophyte produces a range of alkaloid metabolites (Lane *et al.*, 1999; Lane, 1999). Endophyte was shown to be the agent of ryegrass staggers (Fletcher & Harvey, 1981), and lolitrem B was shown to be the major causal factor (Gallagher *et al.*, 1981), although infected ryegrass may contain a number of similar compounds (Miles *et al.*, 1992), some more tremorgenic than others. Lolitrem B is also active against ASW, deterring larval feeding and reducing larval growth rates and survival (Dymock *et al.*, 1989a; Prestidge & Gallagher, 1988). As mentioned above, endophyte deters feeding by adult ASW. Peramine was shown to be the major causal factor (Rowan & Gaynor, 1986). Tall fescue toxicosis is associated with endophyte infection (Hoveland *et al.*, 1980), and the chief toxin responsible is ergovaline (Yates *et al.*, 1985). This compound is also found in infected ryegrass (Rowan *et al.*, 1990), accounting for reports of heat stress of livestock in northern NZ (Easton *et al.*, 1996; Sutherland, 1984) and perhaps interacting with lolitrem B to exacerbate ryegrass staggers (Fletcher & Easton, 1997). Ergovaline is the most abundant of a number of lysergyl compounds produced by endophyte, and these may have a range of pharmacological effects. They have been shown to contribute to ryegrass resistance against black beetle (Ball *et al.*, 1997c). Other less-understood compounds are also produced by endophytes in their host grasses (Lane *et al.*, 2000).

The location of the alkaloid metabolites in the plant reflects in part the location of the endophyte (Ball *et al.*, 1995; Keogh *et al.*, 1996); lolitrem B and ergovaline are found

primarily in the leaf sheath, the true stem, inflorescence and the seed. Ergovaline is particularly elevated in the crown and the inflorescence (Lane *et al.*, 1997a). Peramine moves more freely through the plant shoot, and may have a higher concentration in the leaf blade than the sheath. When the seed germinates, peramine and, to a lesser extent, lolitrem B move through the developing seedling, offering protection against insect attack before the renewed post-germination endophyte activity begins to generate fresh peramine (Ball *et al.*, 1993).

Low amounts of peramine, ergovaline and lolitrem B have been found in root tissue (Ball *et al.*, 1997a; Ball *et al.*, 1997b). There is evidence that some root-feeding invertebrates may be sensitive to endophyte-infection (Eerens *et al.*, 1998b), but there is no conclusive evidence of important effects (Prestidge & Ball, 1993).

Production of peramine, lolitrem B (and similar compounds) and ergovaline (with other lysergyl compounds) are properties of the fungus, not the plant (Lane *et al.*, 1999). However, the plant does exercise some genetic control over the growth of the endophyte within it and the endophyte production of metabolites (Ball *et al.*, 1995; Latch, 1994).

In their physiologic effects on animals, the endotoxins appear to often have a synergistic effect, and may even have additional synergistic effects with *Fusaria* toxins when consumed by grazing animals (Whalley, 2002).

2.5 Variation in Alkaloid Concentrations

Concentrations of peramine, ergovaline and lolitrem B vary in parts of the plant as different tissues age. Thus, concentrations of lolitrem B are greater in older than in younger leaves, while those of peramine are the reverse (Keogh *et al.*, 1996). Endophyte hyphae extend during the period of growth of a particular leaf, but there appears to be no extra growth of hyphae after leaf growth ceases (Schmid & Christenson, 1999). Production of peramine, ergovaline and lolitrem B is maintained when the leaf is mature (after endophyte hyphal growth has ceased), until senescence. As senescence approaches, peramine declines in the leaf, while the concentration of lolitrem B remains high.

The mean concentrations of peramine, ergovaline and lolitrem B in pasture vary with time as the structure of the pasture canopy evolves. In particular, levels increase as the proportion of reproductive tissue in the plant increases.

Environmental conditions also affect metabolite concentrations (Lane *et al.*, 1997b). Water deficit was associated with increased ergovaline and lolitrem B concentrations in both field and growth cabinet. Peramine levels were increased in the field by water stress, in only one of two years. Peramine was not measured in the growth cabinet. Ergovaline concentrations were elevated in large and in small plots receiving extra nitrogen. Lolitrem B levels were higher in the high nitrogen large plots, but the reverse effect was observed in a small plot experiment. No consistent effect of temperature was observed in the growth cabinet experiment.

In a pasture, higher herbage concentrations of lolitrem B and ergovaline have been measured in urine patches than in surrounding areas (Keogh & Clements, 1993). The

importance of this observation is augmented by a tendency of livestock to preferentially graze these areas (Keogh, 1986).

The combined effects of plant development and changing environmental conditions lead to a pattern of change of metabolite levels throughout the year (Ball *et al.*, 1995; di Menna *et al.*, 1992; Easton *et al.*, 1993). Both ergovaline and lolitrem B tend to be in low concentration in early spring, and to increase with rising temperatures and reproductive development, to a first peak at maximum seedhead emergence. The levels fall in the post-reproductive regrowth to rise again through the summer in response to increasing water stress and perhaps temperature, along with an accumulation of older leaf and, under continued grazing, an increase in the proportion of leaf sheath to leaf blade. Concentrations fall again in the autumn, but this is the least predictable period. Prolonged water stress and high temperatures in some circumstances may delay falls in alkaloid levels, and the release of mineralized nitrogen when rain ends an extended dry period (Russell, 1991) may provoke a late peak.

Ergovaline and, to a lesser extent, lolitrem B remain in senescent leaves, and also in leaves killed by defoliation or rapid desiccation. Dead leaf in pasture may therefore be a significant source of both toxins, as may hay. There is some evidence that silage may also retain some toxic potential (Clark *et al.*, 1996).

2.6 Pest Protection & Persistence: A Summary of the Main Events

In the trial established at Ruakura to compare stock performance on infected and endophyte-free pasture, the endophyte-free sward was severely damaged by ASW (Prestidge *et al.*, 1982). Subsequent work established the crucial role played by endophyte in ensuring the persistence of PRG (Table 1) and the reputation of the species as well adapted to NZ. Without endophyte, PRG swards in Waikato, Canterbury and some other districts simply did not persist (Mortimer & di Menna, 1983; Prestidge & Ball, 1993; Popay & Rowan, 1994). Both field plots and glasshouse pot experiments were used in this work.

The resistance of endophyte-infected PRG to ASW is based on deterring the adult weevil from feeding (Barker *et al.*, 1984b). Fewer eggs are laid (Gaynor & Hunt, 1983) so that larval numbers are lower. The compound peramine, produced by the endophyte and translocated through the shoot system, provides this deterrence. Peramine in the seed coat is translocated after germination through the emerging seedling, deterring insect feeding in the early period of growth (Stewart, 1985; Ball *et al.*, 1993).

ASW larvae are not deterred from feeding, but growth rates and survival of larvae are lower on endophyte-infected than endophyte-free PRG (Barker *et al.*, 1984a).

ASW is not the only invertebrate pest sensitive to endophyte. Black beetle (*Heteronychus arator*) is sporadically serious in northern NZ, attacking PRG pastures if they are free of endophyte, and several other grass species. However, infected PRG is not severely attacked (Ball & Prestidge, 1992).

Table 1: Major discoveries of endophyte effects on pasture invertebrates. (From Easton *et al.*, 2001).

Discovery	Reference
Ryegrass infected with wild-type endophyte is resistant to Argentine stem weevil (ASW).	Prestidge <i>et al.</i> (1982)
The ASW feeding deterrent, peramine, is isolated from wild-type-infected ryegrass.	Rowan & Gaynor (1986)
Lolitrems B affects ASW larvae growth and development.	Dymock <i>et al.</i> (1989)
Ergovaline deters adult ASW.	Popay <i>et al.</i> (1990)
Lolitrems-free endophytes give resistance to ASW.	Fletcher <i>et al.</i> (1991)
Black beetle adults are deterred by wild-type endophyte.	Ball & Prestidge (1992)
Ergovaline is identified as a major factor in black beetle resistance.	Ball <i>et al.</i> (1997)
AR1-infected ryegrass is as resistant to ASW as wild-type.	Popay <i>et al.</i> (1999)
Ryegrass with endophyte, including AR1, is resistant to a pasture mealy bug.	Pennell (1998) unpublished data; Popay <i>et al.</i> (2000)
Ryegrass with AR1 has some resistance to black beetle.	Popay & Baltus (2001)

Recent data have revealed the sensitivity to endophyte of the pasture mealy bug (*Balanococcus poae*), and the severe effects this little-known insect can have on endophyte-free PRG pastures, particularly after a dry summer (Pennell & Ball, 1999; Popay *et al.*, 2001).

Other invertebrate research has studied root- and soil-dwelling nematode species (Eerens *et al.*, 1998b; Watson, 1990; Stewart *et al.*, 1993; Watson *et al.*, 1995), several other pasture pests and beneficial soil invertebrates such as earthworms (Prestidge & Marshall 1997; Prestidge *et al.*, 1997). None of these have shown the clear response to endophyte established for ASW, black beetle and pasture mealy bug.

Direct enhancement of PRG growth by endophyte was documented by Latch *et al.* (1985), but other experiments (Barker *et al.*, 1997; Hume *et al.*, 1993) (D.E. Hume, H.S. Easton & M.P. Rolston, unpublished data cited by Easton, 2001) have not shown this. Endophyte-infected PRG competed more aggressively with associated white clover (Sutherland & Hoglund 1989; Stevens & Hickey 1990; Sutherland *et al.*, 1999), and a direct suppressing factor released from grass litter has been suggested. However, most of the effect can be ascribed to greater grass vigour, arising from protection from invertebrate pests and over-grazing (Prestidge *et al.*, 1992).

American scientists have identified pests that are sensitive to endophyte, but do not consider this the major factor in the superior persistence of endophyte-infected fescue. Their research has focused on direct effects of the fungus, on grass growth and drought tolerance. In contrast, it is considered in NZ that pest protection is the primary component of superior productivity and persistence of endophyte-infected PRG pastures (Easton *et al.*, 2001).

The incidence of invertebrate pests varies with regional conditions, and a number of trials have shown no yield or persistence advantages to endophyte-infected ryegrass in Southland (Eerens *et al.*, 1998a). Endophyte-free ryegrass can be used in parts of that region.

2.7 PRG Endophyte & Animal Health Effects (Mainly in Sheep)

Sheep grazing different seedlines of PRG in an experiment at Lincoln were very differently affected by ryegrass staggers. The pasture causing the worst staggers had high levels of infection with endophyte, while the sheep free of staggers were grazing a pasture with almost no endophyte (Fletcher & Harvey, 1981; Fletcher, 1982). This led to an intensive programme documenting and quantifying the effects of endophyte on sheep (Table 2). The precise effects of the staggers toxin on nerve and muscle function (McLeay & Smith, 1999; Munday-Finch & Garthwaite, 1999) have been described, and genetic resistance in sheep has been measured (Morris *et al.*, 1999).

Ryegrass staggers has been responsible for the loss of 900 sheep at a cost of around \$61,000 on an East Coast property (Milne *et al.*, 1999). Deaths, ataxia, depressed intake and liveweight gain, heat stress, dagginess and lowered prolactin levels are all features of endotoxicosis (Bluett *et al.*, 1999; Eerens *et al.*, 1992; Fletcher, *et al.*, 1999, 1993; Fletcher & Sutherland 1993a; b). Presence of clover in the sward may reduce endophyte effects (Eerens *et al.*, 1992).

Table 2: Major discoveries of effects of endophyte-infected ryegrass herbage on health and performance of grazing livestock. (From Easton *et al.*, 2001).

Discovery	Reference
Lolium endophyte causes ryegrass staggers.	Fletcher & Harvey (1981)
Lolium endophyte reduces rate of liveweight gain in lambs grazing PRG.	Fletcher (1983)
Lolium endophyte in PRG depressed serum prolactin in lambs; a link established between ryegrass and fescue toxicosis.	Fletcher & Barrell (1984)
The first lolitrem-free endophyte in PRG evaluated, showing possible elimination of ryegrass staggers.	Fletcher <i>et al.</i> (1991)
Lolium endophyte linked to faecal contamination (dags).	Fletcher <i>et al.</i> (1993); Pownall <i>et al.</i> (1993)
Livestock grazing herbage infected with AR1 endophyte strain, free of ergovaline and lolitrem B, are free of all toxic effects.	Fletcher & Easton (1997)
Sheep can be bred for resistance to RGS.	Morris <i>et al.</i> (1999)
Milk production of dairy cows sometimes affected by endophyte.	Blackwell & Keogh (1999); Clark <i>et al.</i> (1996); Thom <i>et al.</i> (1999)
Metabolites other than lolitrem B in ryegrass/endophyte associations can cause staggers symptoms in grazing livestock.	Fletcher & Tapper, unpublished data (1991, 2001) cited by Easton <i>et al.</i> , 2001.

Ryegrass staggers is the most obvious and immediately serious effect, but many other indicators of sheep health and productivity are affected. Notable among these are the effects on liveweight gain, on serum prolactin levels and on body temperature control. Liveweight gain of lambs, pre- and post-weaning, and of older sheep has been shown to be depressed by grazing endophyte-infected PRG (Bluett *et al.*, 1999; Fletcher & Sutherland, 1993; Watson *et al.*, 1999; Fletcher *et al.*, 1999). Depression in gain of more than 30%, sustained over many weeks, has been recorded for hoggets, and of up to 90% for lambs. In the study of Watson *et al.* (1999), grazing endophyte pasture depressed ewe intake and liveweight, and resulted in reduced growth rates of suckling single and twin lambs.

Serum prolactin concentration in sheep responds to ambient temperature, with levels rising from typically below 50 ng/ml at moderate temperatures, to above 200 ng/ml in warm conditions. Sheep grazing endophyte-infected PRG do not respond in this way, so that at higher temperatures, there is a wide difference between animals grazing infected and endophyte-free herbage (Fletcher *et al.*, 1997). These results conform to published American reports of cattle grazing tall fescue. Depression of serum prolactin is now recognised as a sensitive indicator of exposure of livestock to endophyte-related toxicity.

Animals grazing endophyte-infected PRG frequently show a higher body temperature, particularly when held in conditions of high ambient temperature and humidity, the difference sometimes being a full Celsius degree. Associated with this, sheep grazing endophyte-infected PRG may exhibit severe panting (Fletcher *et al.*, 1999).

Ryegrass staggers is a problem specific to ryegrass pastures, but other endophyte-related symptoms in livestock are similar to some effects of infected tall fescue, with some of the same compounds involved.

Kramer *et al.* (1999) studied the effects of endophyte infected tall fescue on the fertility of Finn x Romney 2-tooth ewes. Groups (n=20) were grazed on either of two lines of endophyte-infected tall fescue, one producing ergovaline (EV+) and the other ergovaline free (EV-) for two weeks and then mated on the treatments. Ovulation rate, conception rate, and numbers of lambs carried were recorded. Levels of serum prolactin and ergovaline in the pasture were determined. Ergovaline levels in the herbage were 3.30 ± 0.60 mg/g and 0mg/g in the EV+ and EV- pastures, respectively. Ewes grazing the EV+ treatment had significantly lower ($P < 0.05$) ovulation rates and number of lambs carried to 90 days of pregnancy than the EV- group. Serum prolactin was significantly ($P < 0.001$) reduced in the EV+ group. The authors concluded that the results indicated that similar effects may occur in ewes grazing endophyte-infected perennial ryegrass pastures containing ergovaline and that further trials are being undertaken to examine this possibility.

Keogh (2002) reports an increased incidence of pneumonia in lambs grazing ryegrass pastures on farms in Northland, which may be linked to the heat stress caused by ergovaline (Keogh pers. comm.).

2.8 Summary of the Main Chemical Compounds

In summary, the wild endophyte present in the perennial ryegrasses in most NZ pastures produces several alkaloids and three of these are significant to stock management:

- Lolitrem B - affects the nerves and muscles of grazing animals, leading to ryegrass staggers.
- Ergovaline - causes heat stress in grazing animals.
- Peramine - deters some insects, particularly ASW, from feeding on the ryegrass. The effects on plant host and animals is summarised in Table 3.

Table 3: A summary of the main alkaloids in endophyte, their occurrence and effects on plant pests and grazing animals

Endophyte Option	Definition Produced	Main Chemicals	Properties
Wild	Naturally infected with an endophyte	Lolitrem B, Ergovaline, Peramine	Protects against ASW and black beetle, can cause ryegrass staggers.
Nil	Contains no endophyte	Nil	No protection against insects, no ryegrass staggers.
AR-1	Novel endophyte	Peramine	Protects against ASW, offers some protection against black beetle but not as much as wild endophyte. No ryegrass staggers.

Techniques for determination of levels of chemicals in samples have improved, in accuracy, in the numbers processed with the same resource, and in the ability to analyse very small samples so that the precise location can be determined (Davies *et al.*, 1997; Spiering, 2000).

2.9 Cattle Research

There has been significantly less work on cattle than on sheep. Depressed serum prolactin has been recorded in cattle grazing endophyte-infected PRG (Blackwell & Keogh, 1999; Easton & Couchman, 1999). It has been argued that heat stress in cattle in northern NZ ascribed to tall fescue was probably often due to endophyte-infected PRG (Easton *et al.*, 1996).

A series of trials conducted by Dexcel (formerly Dairying Research Corporation) showed that any effects on dairy cows were irregular and probably minor (Thom *et al.*, 1999). Clark *et al.* (1999) and Bluett *et al.* (2001) showed endophyte depressed production by 5 to 10% and differed between seasons and years. However, an onfarm study in Northland, following a split herd through a whole season, indicated 20% greater milk production per cow on ryegrass pasture free of endophyte toxins than on naturally infected pasture in the first year (Blackwell & Keogh, 1999) and 5% and 8% greater production in the second and third seasons (Blackwell & Keogh 2001; Keogh & Blackwell, 2001). Seasonal differences were also noted in this study with October and November milk levels the same but depressed in December (Keogh *et al.*, 1999). Australian results with dairy cows were

similar but not unequivocal (Valentine *et al.*, 1993), but the authors recommend against grazing cows on high endophyte ryegrass. The areas and cow numbers involved changed between seasons, and there were different confounding factors at work. The current trials at Dargaville, conducted by Northland interest groups, and at Dexcel will provide more information. Endophyte did not affect growth rates of bulls in a Manawatu study (Cosgrove *et al.*, 1996), although feed intake was affected in one season. A smaller study in Northland showed depression of weight gain of yearling heifers and weaner bulls (mean of 21% in three summer-autumn experiments) and on one occasion, elevated respiration rates (Easton & Couchman, 1999).

While cattle may appear to be relatively tolerant or resistant to endophyte toxins (Bluett pers. comm., Easton pers. comm.), not all studies support his contention. Hamilton-Manns & Crothers (1999) presented case studies of two Manawatu properties that experienced ryegrass staggers (RGS); a dairy farm and a run-off grazing both dairy heifers and intensive bull beef. Before its conversion from a sheep and cattle enterprise to dairying an outbreak of RGS was experienced in 1987. Tall fescue-based pastures were established to minimise the potential threat of RGS. In the intervening 12 years, high endophyte ryegrass cultivars have been established and hotter, drier summers are being experienced in this region. Severe cases of RGS have been observed in three years. On the dairy farm in 1998/99, 21 cows were dried off in January due to severe RGS. The loss in production is impossible to measure. On the run-off property, 16 heifers and weaner bulls died during that summer due to RGS and those alive were 35 kg below target. Control of staggers by using strains of PRG without lolitrem does not always result in a liveweight gain response in cattle, however (Cosgrove *et al.*, 1993).

While visible heat stress is often observed with cattle, statistically significant effects on body temperature have not been recorded (Cosgrove *et al.*, 1996; Blackwell & Keogh, 1999; Easton & Couchman 1999).

Given the very serious effects of tall fescue on cattle performance in trials in USA, it had been expected that effects of ryegrass endophyte on cattle would be more marked. This has raised the possibility that factors other than ergovaline may contribute to the tall fescue situation (Lane *et al.*, 1999).

2.10 Horse Research

Most of the research on horses has been conducted in the USA on tall fescue endophyte. Because of the similarity of the compounds produced by the endophytes of tall fescue and perennial ryegrass, there is a possibility that similar, milder, subclinical effects from PRG endophyte may be experienced with sheep and cattle in NZ. Whalley (2002) summarised much of the horse related research in a recent newsletter for the Waikato Thoroughbred breeders. Whalley (2002) says:

“Our ryegrass staggers is a condition caused by toxins produced by a fungus found in certain cultivars of ryegrass. This fungus or endophyte develops within the plant and releases several toxins, notably lolitrem and ergovaline. These occur in various amounts and at various time, but generally more prevalent from October to May. With most mycotoxins, exposure to as little as a few parts per billion in the

feed can cause severe health problems in horses, young horses and pregnant mares being perhaps most sensitive.

Whilst a large acute dose of some mycotoxins can kill a horse, it is however the chronic sub-lethal doses that potentially are more harmful. Such exposure can cause organ damage specifically to the liver and kidneys, suppressing the immune system which ultimately leads to secondary bacterial infections and general unhealthiness. It is the compromise of the natural immune system that creates difficulties for the horse in withstanding such mycotoxin challenges.

A variety of different symptoms, including reduced body weight gain, increased body temperature, rough hair coat, reduced reproductive performance, reduced birth weight, lower and/or no milk production, and lower serum prolactin levels have been reported in horses consuming endophyte-infected pastures.

Major reproductive problems in horses have been related to endophyte-infected tall fescue. Fescue toxins affect reproduction in both males and females. The majority of reproductive research in horses has focused on the pregnant mare. Agalactia (no milk production) is the most commonly reported clinical sign in mares consuming infected fescue. Other effects in mares include prolonged gestation, thickened placentas, extremely high levels of foal mortality and dystocia. The mechanisms thought to be responsible for reproductive problems are decreased concentrations of prolactin and melatonin, vasoconstriction affecting blood flow to internal organs and hyperthermia”.

Horses seem much more prone to endophyte toxins than sheep and cattle (Bluett pers. comm.).

2.11 Seed Management

Prior to the discovery of the importance of endophyte, different seed lines of PRG had been reported to differ in their resistance to ASW (Kain *et al.*, 1982). Seed lines of the same cultivar were present in commerce with markedly different levels of endophyte infection.

Endophyte in stored seed has been shown to lose viability while the seed itself retains its germinability (Rolston *et al.*, 1986). In particular, endophyte viability is sensitive to the humidity of the store environment. Thus seed stored close to 0°C and 30% relative humidity maintains endophyte viability, while seed held at 5°C and 60% R.H. will suffer a loss of its viable endophyte infection in a few months (Hare *et al.*, 1990; Rolston *et al.*, 1986). As a result of this, before the importance of endophyte was understood in NZ, different seed lines of the same cultivar could have widely differing levels of endophyte infection. Seed falling to the ground and not germinating for several months is also likely to lose viable endophyte (Hume *et al.*, 1999), accounting for the existence of endophyte-free plants in old pastures. Where insect pressure is significant, natural selection eliminates these plants from the sward, but in southern NZ they survive (Widdup & Ryan, 1992).

Endophyte dies in stored seed unless the storage conditions are carefully controlled (Rolston *et al.*, 1986). There can be catastrophic loss of endophyte viability in a few months if humidity is high. Critical upper limits in short term bulk storage for temperature, relative humidity (RH) and seed moisture are 10°C, 50% and 11% respectively. Seed stored below 5°C, 40% RH and 11% seed moisture has maintained viable endophyte levels for up to 15 years.

PRG seed crops are regularly treated with fungicide to control rust and other diseases. At the recommended rates, this use of fungicide does not affect seed endophyte infection (M.P. Rolston, 2000-2001 unpublished data cited by Easton *et al.*, 2001), but as new fungicide products are introduced to the industry, they need to be checked.

Fertiliser management of seed crops can also affect the endophyte status of the harvested seed, with a carryover effect in the subsequent sown swards (Stewart 1986).

2.12 Endophyte Biology

The ryegrass endophyte is closely related to the choke fungi (*Epichloe spp*) (Easton, 1999). These live endophytically within the grass shoot like the *Neotyphodium spp*, but during their sexual stage, form a dense stroma around the reproductive stem of the host below the developing seed head, preventing normal maturation. The *Neotyphodium* endophytes do not develop this visible and dramatic expression, and have no sexual stage. They are not visible in the grass, and have no means of propagation between plants.

The endophyte grows in and around the basal meristem of grass tillers, and in each newly forming leaf. As the new leaf forms, with the blade and the sheath, the endophyte grows to a slight extent into the blade, and much more abundantly into the leaf sheath (Schmid & Christensen, 1999). Hyphae elongate as the leaf sheath extends. There appears to be no extra growth of hyphae within a leaf after leaf growth ceases. When a tiller becomes reproductive, the endophyte grows within the elongating stem, into the developing inflorescence, the flower and the developing seed and the embryo as it forms (Philipson & Christey, 1986). At germination, the endophyte hyphae in the seed but outside the embryo appear to play no further part, while those within the embryo grow with the seedling and thus the new plant.

Because of the growth pattern described above, endophyte is found concentrated in the leaf sheath, particularly near the base, in the reproductive stem and the inflorescence. Concentration is relatively low within the leaf blade (Keogh *et al.*, 1996). Traces have been detected in ryegrass roots (Musgrave, 1984). Tall fescue endophyte was thought not to be found in the roots (Hinton & Bacon 1985), but hyphae have been detected (Azevedo & Welty, 1995).

Parallel with field studies has been work on the basic biology of the fungus and its interaction with its host grass. The endophyte species commonly present in PRG were characterized (Latch *et al.*, 1984), and the variation (biodiversity) among endophyte strains found in different ryegrass and fescue species and populations has been analysed in NZ and abroad (Christensen *et al.*, 1991; Christensen *et al.*, 1993). Field work was facilitated by rapid immunological detection of the fungus (Musgrave, 1984). Growth within the plant,

and into the developing seed for transmission to the next grass generation was described (Philipson & Christey, 1986), and the co-ordination of endophyte and host growth was highlighted using a genetically transformed laboratory strain to precisely locate endophyte metabolic activity (Schmid & Christensen, 1999; Spiering, 2000). The biochemical pathways leading to the production of lolitrem B are being elucidated (Scott *et al.*, 1999).

2.13 Selection of Non-Toxic Endophyte Strains

The different alkaloids have separate, independent biosynthetic pathways, allowing the opportunity to select for or develop endophyte strains with different or nil levels of the particular alkaloid (Latch, 1994).

Knowledge of the endophyte and its host has enabled a programme to manipulate and manage the association. There had been a long record of research on pasture management to minimise ryegrass staggers, and endophyte research was related back to this. The precise location in the plant of the fungus and of the different compounds it produces (Keogh *et al.*, 1996), and the effects of different environmental variables on the fungus and its activity (Lane *et al.*, 1997) have been identified. This has enabled a refining of management practices (Keogh & Clements, 1993).

The fungus can be isolated and grown in culture, and young seedlings can be artificially infected with it (Latch & Christensen, 1985). Endophyte strains isolated from PRG in NZ were largely similar in their chemistry, but some strains isolated from overseas PRG did not produce all the major compounds (Davies *et al.*, 1993; Latch, 1994). Transfer of strains to a different host showed that their properties to produce or not produce particular compounds were stable. However, the host plant can affect the amount of the compounds produced by the endophyte (Easton, Latch, Tapper & Ball 2002 *in press*). Strains vary in their ability to form effective associations with a new host (Christensen *et al.*, 1997), involving controlled growth into all new shoot meristems, efficient transmission into seed and good viability in stored seed. Ineffective strains may fail at any or all of these points. Likewise, plants vary in their suitability as a host for a transferred strain. Efficiency of inoculation and seed transmission, and production of compounds may all vary for one strain in different plant material (Easton *et al.*, 2001).

2.14 Selected Strains In The Field

Endophyte strains from PRG have been identified which produce peramine but are free of ergovaline or lolitrem B or both (Fletcher *et al.*, 1991; Latch, 1994). "Endosafe" was released as a strain that produces no lolitrem B. This was the strain referred to in the literature as 187 (Latch, 1994; Easton, pers. comm.). It had supported apparently healthy sheep with no ryegrass staggers, and allowed ryegrass to grow and persist well (Fletcher *et al.*, 1991). However, in PRG, it produced ergovaline at levels higher than those found in naturally infected certified PRG cultivars. This was reflected in depressed liveweight gain, relative to sheep grazing endophyte-free PRG, elevated body temperatures and elevated respiration rates (Fletcher & Easton, 1997). It is no longer available in PRG, but has been retained in the market in the hybrid ryegrass "Greenstone" where the level of ergovaline is relatively low, and no livestock health problems have been encountered (Easton *et al.*, 2001).

A recent development is the novel endophyte (AR1) produced by AgResearch, and it has been licensed to seed companies for use in commercial ryegrasses. AR1 is a 2nd generation endophyte that significantly reduces the risk of animal health problems associated with the high endophyte ryegrasses. Trials at Lincoln showed animal performance on AR1 endophyte pastures to be similar to those on endophyte-free pasture.

AR1 strain, a second generation endophyte, is free of both ergovaline and lolitrem B, and its effects on PRG agronomy and the health and productivity of grazing livestock have been intensively studied (Fletcher & Easton, 1997; Fletcher, 1999; Popay *et al.*, 1999; Fletcher & Easton, 2001). Sheep growth rates, lambing percentages, serum prolactin profiles, body temperatures, respiration rates and dag burdens are all the same as for sheep grazing PRG free of endophyte (Table 4). Mean herbage yield over several sites throughout NZ is significantly better for PRG infected with AR1 than for endophyte-free PRG (Popay *et al.*, 1999). AR1 provides excellent protection against ASW. However, the protection provided against black beetle by AR1 is not always as robust as that provided by the naturally occurring endophyte (Popay & Baltus, 2001).

Table 4: Effects of ryegrass infected with selected endophyte strain AR1 on grazing sheep, relative to the same ryegrass cultivar infected with naturally occurring toxin-producing endophyte (WT) or free of endophyte (Nil) (results of Fletcher, 1999 cited by Easton *et al.*, 2001).

		WT	Nil	AR1
Lambs	LWG g/hd/day	23 ^a	120 ^b	131 ^b
	Rectal temp °C	40.5 ^a	40.0 ^b	40.1 ^b
	Respiration/min	97 ^a	73 ^b	79 ^b
	Serum prolactin ng/ml	96 ^a	185 ^b	203 ^b
	RGS, scale 1-5	3.2 ^a	0 ^b	0.3 ^b
Hoggets	LWG g/hd/day	165 ^a	191 ^b	212 ^b
	Serum prolactin ng/ml	101 ^a	333 ^b	344 ^b
	Dags, scale 1-5	1.4 ^a	0.6 ^b	0.4 ^b

^{a, b} Means in the same row with different superscripts are significantly different at the 4% probability level.

Meat NZ has played an ongoing key role in the development of safe endophytes and seed multiplication of new non-toxic endophyte/grass associations, but the seed multiplication phase prior to grazing trials is a persistent bottle neck (Meat NZ, 2000/01).

The NZ seed industry has maintained an active interest in endophyte research, and has funded significant elements of it (Easton *et al.*, 2001). In 1999 Warwick & Green (1999) stated:

“The NZ Plant Breeding and Research Association and AgResearch have entered into an agreement to commercialise AR1, a new selected endophyte with high peramine, low lolitrem and low ergovaline levels. A number of commercial cultivars have been inoculated and these are now going through an agreed validation

process with animal performance and agronomic trials being conducted throughout NZ. The seed industry is not currently in a position to confirm a commercialisation date, but it will not be before 1 January 2001”.

Selected endophyte is now reasonably freely available to NZ farmers through seed of the PRG cultivars developed and marketed by the different companies. AR1 was released to farmers in limited quantities in ten commercial ryegrass cultivars in autumn 2001 (Meat NZ 2000/01).

Establishment and management of pastures with selected endophyte, free of plants carrying toxic endophyte, requires preparation to minimise the load of buried seed (Bluett, *et al.*, 2001; Hume & Lyons, 1992; Hume *et al.*, 1999; Hume *et al.*, 2001), and thorough field and seedbed preparation (van Vught & Thom, 1997; Burggraaf & Thom, 2001). An initial modest presence of wild-type contaminants among the seedlings may increase under favourable natural selection (Francis & Baird, 1989; Hume & Brock, 1997; Burggraaf & Thom, 2001). Established pastures may be contaminated with seed from hay fed onto the pasture, or faeces of animals that have recently grazed toxic pastures with mature seedheads (van Vught & Thom, 1997; Burggraaf & Thom, 2001). A proportion of PRG seed passed in faeces between 10 and 36 hours after ingestion proved to be viable and to contain viable endophyte (Rolston *et al.*, 2001). To avoid contaminating a field sown to PRG infected with selected endophyte, these authors recommended that stock have no access to PRG hay or PRG pasture with mature seedheads in the 36 hours prior to entering.

2.15 Italian & Hybrid Ryegrasses

The endophyte species characteristic of PRG is not normally found in Italian ryegrass. Another endophyte species (*N. occultans*) is present in Italian ryegrass (Latch *et al.*, 1988). It is much slower growing than the PRG endophyte, and does not proliferate as abundantly through the host tissue. There is evidence that the young seedling is protected from invertebrate attack (Stewart, 1987; Piggot *et al.*, 1988), but the *N. occultans* appears to have no effect on established pasture or on grazing livestock (Piggot *et al.*, 1988; Prestidge, 1991).

Perennial and Italian ryegrasses freely hybridise, and hybrid seed developed on the perennial parent may be infected with *N. lolii*. Hybrid ryegrass populations and cultivars may thus be infected with the PRG endophyte, and hybrid populations similar to Italian ryegrass have been identified which are infected with the PRG endophyte (Piggot *et al.*, 1988).

2.16 Tall Fescue

While the focus of NZ research has been PRG, the release of a selected non-toxic tall fescue endophyte (“Max-Q”) in the USA, in partnership with University of Georgia and other American interests, is a successful byproduct of our research. No typical fescue toxicity symptoms have been reported with animals grazing endophyte-free “Demeter”, the most widespread cultivar of tall fescue sown in Australia (Latch, 1994). Endophyte-free tall fescue is valuable in specific situations in NZ (Fraser & Lyons, 1994; Milne *et al.*,

1997), but persistence and performance would be enhanced by an endophyte (Easton & Cooper, 1997). Tall fescue endophyte produces compounds other than those causing fescue toxicosis. Information to date indicates that “Max-Q” supports excellent livestock production in the USA (Bouton *et al.*, 2001), and in one NZ experiment (Fletcher *et al.*, 2001).

Meat NZ funded the first on-farm trials with a safe endophyte in tall fescue. These were established in autumn 2001 (Meat NZ, 2000/01).

2.17 Control & Prevention of Endotoxicoses

Adsorbent Compounds

Theoretically compounds which can absorb or bind the agent in the digesta in lab studies may reduce the toxic effects (Whalley, 2002; Moorehead, 2002) and these have been used with horses, but have not been proven to be efficacious.

Mycosorb™ from Altech is a unique formulation of esterified glucomanans (EGM) derived from the cell wall of the yeast *Saccharomyces cerevisiae* (Yea-Sacc). The glucomanans have a tremendous surface area and the ability to strongly bind and absorb mycotoxins within the digestive system, so that they are rendered inactive and pass through the digestive system unabsorbed.

Whilst the use of Mycosorb™ specifically for Grass Staggers has not yet been subjected to full scientific evaluation, anecdotal evidence from current and past users suggests quite strongly, a marked affect in helping to minimise the risk, and justifies its inclusion in the diet to horses exposed to pasture endophyte (Whalley, 2002).

Another similar product Biomos™ is produced by Nutritech (Nutritech, 1996). This is a similar product, mannan oligosaccharide, also derived from cell walls of certain strains of yeast. It reputedly also binds aflatoxins and zearalenone. The brochure claims a significant improvement in stock health has been reported in 5-7 days. Dose rates of 20 g/500 kg liveweight for 5-7 days for severe toxicosis reducing to 10 kg/500 kg LW, and 10 kg/500 kg LW for mild cases, reducing to 5 kg/500 kg LW. This is for horses, cows and other ruminants.

Clay compounds such as zeolite and bentonite (Moorehead, 2002; Towers pers. comm.) have also been used, but no success stories have been reported.

Other Remedies

There are anecdotal accounts of other remedies such as cider vinegar, KCl/MgSO₄, Nutrimol™, seaweed extract and trace element/vitamin E supplements being used (Milne *et al.*, 1999; Whalley, 2002), but again generally with low success. Theoretically these supplements should help boost the immune system.

Drugs

Bomperidone is used successfully to treat horses, especially brood mares with fescue toxicosis in the USA (Fletcher, pers. comm.).

Immunisation

Thompson *et al.* (1993) report that passive immunisation with monoclonal antibodies, specific for ergopeptine alkaloids has reversed signs of fescue toxicosis in grazing steers. I have found no other literature on the subject, but the array of toxins produced by endophytes could make this a long hard road to success.

Genetic Selection

Morris *et al.* (1999) report on a six year selection response for resistance or susceptibility to ryegrass staggers (RGS) in sheep. There has been a reasonable response with a 24% difference in clinical incidence between the two lines. The same authors report that a FE resistant line of sheep was also resistant to RGS. Their explanation is that sporidesmin and lolitrem may have a common biochemical pathway, but I believe they may have enhanced the detoxifying capacity of the liver. It would be interesting to see if these lines were able to tolerate toxic ergovaline levels. If it is a liver function explanation, it may be worth pursuing, but if selection for individual toxins was required, this would also be a long drawn out approach.

Grazing Management

Reducing the intake of perennial ryegrass containing the wild endophyte is the only prevention only. The obvious thing is to dilute the ryegrass intake with supplements or remove the animals on to a ryegrass free pasture (Keogh, 2002) or crop (Milne *et al.*, 1999). Webby *et al.* (2001) used alternative feeds, silage and forage crops, to reduce intake of fungal toxins with a group of 12 farmers in West Waikato with some success.

The recommendations for the control of clinical staggers are that they should be removed from all perennial ryegrasses containing the wild endophyte. Supplementary feeding with hay and grain may be required. Occasionally ryegrass in hay can contain the mycotoxin so lucerne or clover hay is preferred.

Grazing management can also reduce the intake of the toxic parts of the ryegrass. There are a few practices in the grazing of the toxic ryegrass pasture that may reduce the intake of toxic parts. Keogh & Clements (1993) recommend a rapid rotation of daily shifts as a RGS control procedure. Neurotoxin intake was substantially less on day 1 than on days 2 and 3 of a grazing period; and the aim is to prevent regrazing previously grazed areas.

Moorehead (2002) says: "Management of pastures to prevent horses from eating the ryegrass with the high concentrations of endophyte is difficult. Topping or cross grazing with cattle to decrease the amount of seed head is recommended".

Replacing Toxic Ryegrasses

The most reliable way of avoiding endophyte toxicosis is to replace the toxic ryegrass. AR1 endophyte ryegrass is the obvious choice. Bluett *et al.* (2001) made quite a comprehensive study of resowing strategies. The experiment was conducted at Dexcel, Hamilton from September 1999 to April 2001 to evaluate how pre-sowing pasture management and establishment method influenced the contamination of a newly sown AR1 endophyte-infected ryegrass dairy pasture with ryegrass infected with the wild endophyte (*Neotyphodium lolii*). Contamination level was estimated by counting the number of volunteer ryegrass plants between drill rows and by analyzing bulk ryegrass samples for lolitrem B concentration. Hay, silage, grazed, grazed/topped and a turnip crop as pre-sowing managements generated large differences in viable ryegrass seed density

(2555, 747, 348, 391 and 25 seeds/m², respectively) on the soil surface after natural reseeding in March 2000. Measurements on 20 November 2000 showed the spray/cultivation and double-spray/fallow establishment methods were effective in reducing contamination with volunteer ryegrass plants (8 and 34 plants/ m², respectively), in contrast to drilling AR1 endophyte-infected ryegrass seed into hard-grazed existing pasture (581 plants/ m²). On 14 March 2001, lolitrem B levels were lower in the spray cultivated and double-spray/fallow treatments compared to the hard-grazed treatment (0.3, 0.5, 1.1 µg/g, respectively).

The spray/cultivation or double spray methods are recommended, but there are concerns that these methods cannot be used on hill country (Easton, Fletcher, Widdup pers. comm.; Meat NZ, 2000/01). However, the on-farm work that WoolPro's Sheep Production Officer, Sally Hobson, should help allay these fears (Hobson, 2002). It will be interesting to ascertain the success of the processes on the various farms in the project.

Persistence of the AR1-ryegrass and ingress of wild type and risks that will need to be managed and current Meat NZ (2000/01) projects are examining best management practices required.

Hume (1999) reviewed how new pastures may become contaminated with wild type endophyte ryegrass. This review outlined possible pathways by which new pastures may become contaminated with ryegrass infected with toxic wild-type endophyte. Prevention of contamination by good preparation of the paddock before sowing is a key factor, as it is in general for the successful establishment of any pasture (Hume & Fraser, 1985; Milne *et al.*, 1993). Preparation should:

- Kill all existing vegetative ryegrass plants either by repeated cultivations and/or herbicides.
- Prevent natural reseeding.
- Deplete any buried ryegrass seed through cultivation or time.
- Prevent seed entering via dung, hay/straw, sown seed or machinery.
- Provide a seedbed that favours rapid establishment of the sown pasture (e.g. fertile, moist, fine, weed-free) as this will also reduce the proportion of contaminating ryegrass in the new pasture.

The time taken and methods to achieve this will vary. Evidence from a number of cases indicates that a paddock preparation of two years may be needed to ensure that natural reseeding is prevented for two seasons and that buried seed is reduced particularly in summer-dry environments. Contamination after sowing can be reduced by preventing seed entering via hay/straw, dung or machinery.

Meat NZ has funded work to examine on-farm long-term persistence of non-toxic endophytes (Meat NZ 2000/01).

Fletcher (1999) calculated gross margins for a sheep systems trial of \$605/ha and over \$700/ha, respectively for wild type and AR1 treatments. No conclusive studies have been done with cattle.

2.18 Conclusion

Ryegrass pastures in NZ are usually infected with the ryegrass endophyte (*Neotyphodium lolii*). The endophyte provides protection from invertebrate attack, discourages overgrazing by livestock and perhaps confers other advantages enhancing ryegrass persistence. The endophyte life cycle is completed within the plant, and transmission is uniquely through the grass seed. Within the plant, the endophyte synthesises a range of compounds, some of which have been identified as responsible for known endophyte effects on pasture invertebrates and on livestock. The concentrations of these compounds vary in different parts of the plant, with plant age and in response to environmental influences. Some, especially peramine protect the plant from insect attack, but others have deleterious effects on stock and productivity. Lolitrem B, paxilline and probably a number of unidentified compounds act as neurotoxins causing ryegrass staggers. Ergovaline causes heat stress and lowered blood prolactin levels (with a host of side effects). Others or a combination of these causes scouring and dags in sheep, depressed intake and liveweight gain.

While endophyte has these well-known adverse effects on livestock, it contributes positively to grassland productivity in NZ in a very useful symbiosis with ryegrass.

Recent revolutionary advance in our understanding of PRG pastures and the place of endophyte in them has impacted on pasture use and on seed inventory management. A new product, AR1, offers farmers greatly enhanced livestock production, certainly with sheep, and probably with cattle, particularly in districts where black beetle pressure is not serious. This represents a major achievement. The "Endosafe" strain in PRG does not cause ryegrass staggers and would provide better protection against black beetle than AR1, but livestock performance would not be as good. There remains the goal of better protection of the host grass, but with livestock performing to their potential. Ongoing research programmes have this aim. Meat NZ has a key role in funding this research.

3.0 CONCLUSIONS

- The role of endophytes in a symbiotic plant protection role and as a source of endotoxins detrimental to stock health is now well understood and documented.
- A number of safe endophytes have been developed and one (AR1) has now been commercially released in ten cultivars of ryegrass.
- When AR1 is grazed by stock, none of the ill health or depressed productivity symptoms occur.
- The opportunity for widespread use of this endophyte throughout the NZ pastoral industries, especially the sheep industry, is huge.
- Extension efforts on effective regrassing of hill country with AR1-pastures should be escalated.

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