Ryegrass endophyte: a New Zealand Grassland success story

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Abstract

The discovery in 1981 that ryegrass endophyte is responsible for ryegrass staggers has required a total revision of our understanding of ryegrass-based pastures. The effects on grazing livestock range from obvious clinical disorders to chronic impairment of productivity. On the other hand, endophyte infection is essential to ryegrass persistence in most of New Zealand, protecting the plant from some invertebrate pests and from over-grazing, and perhaps promoting its tolerance of moisture stress. Vigorous, endophyte-infected pastures sometimes include less clover than pastures relatively free of endophyte. Active compounds produced by the endophyte have been identified, and conditions causing high levels of them have been documented in part. Endophyte strains which do not produce the compounds responsible for livestock toxicoses offer exciting new possibilities in grassland agriculture.

Keywords: invertebrate pests, mycotoxicoses, *Neotyphodium lolii*, perennial ryegrass, ryegrass staggers

Introduction

Perennial ryegrass (PRG) is the most widely-sown species in New Zealand 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, a redoubtable toxic weed dominating our roadsides and other waste places.

The endophytic fungi in PRG and tall fescue had been described in 1933 (Sampson 1933) and further studied in the 1940s by New Zealand 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.

The crucial initial discoveries were serendipitous. Since then, endophyte research has been focused and multidisciplinary, but founded on the experience and expertise already present in our research groups. Another feature of the work has been the importance of international contacts, and in particular the close and very fruitful interchange with research groups in the south east of the USA.

Ryegrass staggers and animal health

Sheep grazing different seed-lines 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 1). 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 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 (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.

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 (see below).

Pest protection and persistence

In a 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 2) and the reputation of the species as well adapted to New Zealand. 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 have been 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).

Table 1 Major discoveries of effects of endophyte-infected ryegrass herbage on health and performance of grazing livestock.

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 *et al.* (1991)
Lolium endophyte linked to faecal contamination (dags)	Fletcher (1993); Pownall *et al.* (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 *et al.* (1999)
Milk production of dairy cows sometimes affected by endophyte	Blackwell & Keogh (1999); Clark *et al.* (1996); Thom *et al.* (1999)
Metabolites other than lolitrem B in ryegrass/endophyte associations can cause staggers symptoms in grazing livestock.	Fletcher & Tapper, unpublished data (1991, 2001)

Table 2 Major discoveries of endophyte effects on pasture invertebrates.

Discovery	Reference
Ryegrass infected with wild-type endophyte is resistant to Argentine stem weevil (ASW)	Prestidge *et al.* (1982)
The ASW feeding deterrent, peramine, is isolated from wild-type-infected ryegrass	Rowan & Gaynor (1986)
Lolitrem B affects ASW larvae growth and development	Dymock *et al.* (1989)
Ergovaline deters adult ASW	Popay *et al.* (1990)
Lolitrem-free endophytes give resistance to ASW	Fletcher *et al.* (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 *et al.* (1997)
AR1-infected ryegrass is as resistant to ASW as wild-type	Popay *et al.* (1999)
Ryegrass with endophyte, including AR1, is resistant to a pasture mealy bug	Pennell (1998) unpublished data; Popay *et al.* (2000)
Ryegrass with AR1 has some resistance to black beetle	Popay & Baltus (2001)

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

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) 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, we consider in New Zealand that pest protection is the primary component of superior productivity and persistence of endophyte-infected PRG pastures.

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.

Chemical compounds

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). 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).

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 (B.A. Tapper, unpublished data) (Davies *et al.* 1993; Spiering 2000).

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 was argued that heat stress in cattle in northern New Zealand ascribed to tall fescue was probably often owing 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). However, an on-farm 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). 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 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).

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 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, 50% 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), 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).

Endophyte biology

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 characterised (Latch *et al.* 1984), and the variation (biodiversity) among endophyte strains found in different ryegrass and fescue species and populations has been analysed in New Zealand 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 high-lighted 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).

Selection of non-toxic endophyte strains

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 New Zealand 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).

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. 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.

AR1 strain 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 3). Mean herbage yield over several sites throughout New Zealand 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 3 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)).

		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 5% probability level.

The New Zealand seed industry has maintained an active interest in endophyte research, and has funded significant elements of it. Selected endophyte is available to New Zealand farmers through seed of the PRG cultivars developed and marketed by the different companies.

Establishment and management of pastures with selected endophyte, free of plants carrying toxic endophyte, requires preparation to minimise the load of buried seed (Hume & Lyons 1992; Hume *et al.* 1999; Hume *et al.* 2001), and thorough field and seed-bed 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.

Italian and 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).

Tall fescue

While the focus of New Zealand 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 by-product of our research. Endophyte-free tall fescue is valuable in specific situations in New Zealand (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 New Zealand experiment (Fletcher *et al.* 2001).

If these results are confirmed, selected non-toxic endophyte may greatly enhance the usefulness of tall fescue in New Zealand.

Conclusion

The 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. Clearly there remains the goal of better protection of the host grass but with livestock free to perform to their potential. The accumulated information, and our experience with endophyte strains, arm us well to pursue this goal.

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