# Endophyte in New Zealand ryegrass pastures, an overview

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## Abstract

Ryegrass pastures in New Zealand 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. While endophyte has well-known adverse effects on livestock (reviewed elsewhere in this volume), it contributes positively to grassland productivity in New Zealand.

**Keywords:** endophyte, *Lolium*, *Neotyphodium lolii*, pest resistance, ryegrass

## Endophyte in New Zealand pastures

The natural condition of perennial ryegrass (*Lolium perenne*) is to be infected with the endophyte, *Neotyphodium lolii*. Other endophytic fungi are regularly found in perennial ryegrass (Latch *et al.* 1984). Their influence 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 New Zealand 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 New Zealand sixty years ago (Neill 1940), but an early investigation concluded it had no impact on livestock health (Cunningham 1958). 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 infected at very high frequency. However, in the south of New Zealand, 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 New Zealand (Widdup & Ryan 1992).

## Why is endophyte so widespread in New Zealand?

Endophyte is essential to the thrift of perennial ryegrass pastures in New Zealand 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.* 1984c; Rowan & Gaynor 1986), less eggs and larvae are present in infected swards (Gaynor & Hunt 1983), those present survive less well (Barker *et al.* 1984b) 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.* 1984a). Yield responses of 50–60% in mixed ryegrass–white clover swards were achieved by protecting from ASW with insecticide (Kane *et al.* 1982). In 1991, pasture damage by ASW was estimated as costing New Zealand $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 (Kane *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 New Zealand (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 endoparasitic, 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.* 1998d; Stewart *et al.* 1993). There is very little New Zealand information on the importance of grass-associated nematodes in pastures (Watson *et al.* 1986; Yeates & Prestidge 1986). Pasture mealy bug (*Balanacoccus 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, pers. comm.). 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 New Zealand 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.* 1998a; Hume *et al.* 1993); Easton & Rolston, unpublished data; Easton & Hume, unpublished data). 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 unpublished data). Other field experiments have indicated no such effects (Eerens *et al.* 1998b; 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 (R.H.Watson, pers. comm.). 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 New Zealand (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.

## Basic biology of endophyte

The ryegrass endophyte is closely related to the choke fungi (*Epichloë spp*). 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 seedhead, 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).

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 New Zealand, 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 (however, see 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 New Zealand they survive (Widdup & Ryan 1992).

## Alkaloid metabolites produced by endophyte

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 discussed 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 New Zealand (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.* 1999).

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.* 1998d), 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).

## 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 the 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 through 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 mineralised nitrogen when rain ends an extended dry period (Russell 1991, p. 289) 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).

## Costs and benefits

Later papers in this volume detail the adverse impact endophyte can have on livestock production. Ryegrass staggers, reduced liveweight gain, increased dag burden and fly strike, heat stress, perturbed reproductive performance, depressed milk production have all been noted with varying regularity.

To this list of adverse effects can be added a trend for poorer clover growth in endophyte-infected pasture (Stevens & Hickey 1990; Sutherland & Hoglund 1989). The effect is not always observed (Eerens *et al.* 1998b), and is mostly an inverse effect of ryegrass vigour (Prestidge *et al.* 1992). Direct inhibitory effects on white clover have been documented (Sutherland *et al.* 1999), but apparent stimulatory effects have also been noted (Eerens *et al.* 1998c). 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 in 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 will 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.

## Conclusion

Evidence that reduced grazing intensity occurs when ryegrass is infected with endophyte, may account for some but perhaps not all the weight-gain effects reported (Edwards *et al.* 1993). In terms of plant defence, the toxicity to livestock associated with endophyte infection should be seen as in the same category as resistance against invertebrates. Perennial ryegrass, like tall fescue and other related grasses, has evolved over a long period in association with endophyte. The alkaloid metabolites produced by the endophytes protect the plant from attack by insects and other invertebrates, and discourage over-grazing by mammals.

Monocultures of ryegrass are a product of modern agriculture, and with high use of nitrogen fertiliser, are maintained more completely in western Europe than in New Zealand. However endophyte, by maintaining ryegrass vigour, encouraging selective grazing of companion species and perhaps by directly suppressing companion species, maintains ryegrass as a more dominant species than uninfected ryegrass in the same conditions.

Before 1980, research on ryegrass ignored the specific role of endophyte. Most of this research can now be seen as applying to the association of ryegrass and endophyte. In some cases however, differences in endophyte infection levels between treatments make proper interpretation of the results very difficult. It is now possible to research and manage the components of the association independently. Comparison between ryegrass of different origin is made with all entries at the same level of endophyte infection, and the influence of endophyte can be determined using the same ryegrass infected at different levels. Where it is desirable, it will be possible to identify ryegrass which is adapted to the absence of endophyte, and this is in fact what has occurred for tall fescue sown for pastures in New Zealand (Anderson 1975). Endophyte strains can be identified with particular properties (Latch 1994), and ryegrass strains will be identified with favourable moderating effects on the endophyte (see Adcock *et al.* 1997 for tall fescue data). While optimism concerning genetic enhancement of other symbionts, such as rhizobia and VA mycorrhiza, has not been justified, the ryegrass (and tall fescue) endophytes offer a more favourable scenario because the host grass can not be invaded by volunteer endophyte strains.

It is a quirk of modern globalisation that the ryegrass-endophyte association, which evolved in Europe and north Asia, should be of particular importance in New Zealand, largely because of the presence here of ASW and black beetle, insects from South America and Africa.

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