Endophytes for improving ryegrass performance: Current status and future possibilities

Alison J Popay [A] and David E Hume [B]

[A] AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand. www.agresearch.co.nz
[B] AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North 4442, New Zealand
Contact email: Alison.popay@agresearch.co.nz

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Introduction

The endophyte *N. lolii* was introduced naturally into New Zealand and Australia when perennial ryegrass seed was brought into these countries. Although the presence of the endophyte was recognised early in the 19th Century, its effects were only discovered in the early 1980s when it was found that these Wild-type strains of endophyte caused ryegrass staggers, a neuromuscular condition of grazing animals (Fletcher and Harvey 1981), and that they also protected their hosts from the effects of Argentine stem weevil (*Listronotus bonariensis*) a serious pest of ryegrass in New Zealand (Prestidge *et al*. 1982). These endophytes form systemic infections in the above-ground parts of their host plants, have no external stage and are maternally transmitted in seed. They produce alkaloids which have effects on herbivores, including sheep, cattle and invertebrates. Although all the alkaloids known to be produced by the endophytes have bioactivity against insects, not all are toxic to livestock. Research in New Zealand has focused on exploiting these endophytes for their natural biological control properties while minimising any effects on livestock.

Development of new endophytes

Knowledge of the chemistry of the endophytes was initially the key to development of new endophytes. Three alkaloids were identified as being produced by *N. lolii*, viz. peramine, ergovaline and lolitrem B. All three alkaloids have some effect on Argentine stem weevil but only the latter two are mammalian toxins (Thom *et al*. 2012). In native habitats of perennial ryegrass in Europe, strains of endophyte were found which produced peramine but neither ergovaline nor lolitrem B. For endophyte strains with this alkaloid profile, peramine was sufficiently deterrent to adult and larval Argentine stem weevil to reduce the damage by this insect to the same extent as Wild-type strains (Popay *et al*. 1999) with no detrimental effects on sheep or cattle (Thom *et al*. 2012). Such endophytes also protect against pasture mealybug (*Balanococcus poae*), another regional pest in New Zealand (Pennell *et al*. 2005).

In 2001 an endophyte known as AR1which produces only peramine was commercially released to New Zealand farmers. This endophyte had met the original aims of the research, namely to provide perennial ryegrass with resistance to Argentine stem weevil without causing ryegrass staggers or heat stress in sheep or cattle. It improved animal performance including milk production and was quickly adopted by New Zealand farmers (Milne 2007). Other strains of endophyte were included in trials that preceded the release of AR1. Among these was AR37, an endophyte which produced none of the known alkaloids. This endophyte had no effect on adult Argentine stem weevil but reduced larval damage to low levels (Thom *et al*. 2013). Unlike AR1, it deters adult African black beetle (*Heteronychus arator*) leading to reduced populations of the damaging root-feeding larvae (Thom *et al*. 2013), a serious pest in northern New Zealand and parts of Australia. AR37 also reduces populations of a root aphid (*Aploneura lentisci*), pasture mealybug, porina larvae (*Wiseana cervinata*) (Popay *et al*. 2011) and pasture tunnel moth (*Philobota* spp) (Moate *et al*. 2012). AR37, however, causes some ryegrass staggers although episodes tend to be less severe, more transient and occur less frequently than with the Wild-type (Thom *et al*. 2012). Furthermore, AR37 has no adverse effects on lamb growth or milk production in dairy cows (Thom *et al*. 2012). AR37 was commercially released in 2007.

Ryegrass performance

Endophyte infection is naturally selected for in pastures with many incidences recorded of increasing frequency of infection often over short periods of time (Popay and Hume 2011). This in itself is indicative of an advantage to endophyte-infected plants. Improved plant performance is also reflected in improved yields. In a national series of trials, yields of ryegrass infected with AR1 endophyte often matched that of the Wild-type strain and exceeded that of endophyte-free ryegrass, particularly in the summer-autumn period (Popay *et al*. 1999). In two trials in the northern North Island, annual yields of AR1 and Wild-type-infected ryegrass were similar whereas AR37 exceeded these two treatments by 16 and 18% at Hamilton over 4 years and 47 and 37% at Kerikeri over 3 years (Hume *et al*. 2007). Yield differences were greater during the summer-autumn period and increased with time. AR37 had higher tiller densities than AR1 and Wild-type in this trial and also in the third year of a paddock-scale trial in the Waikato when tiller densities of AR37 exceeded that of AR1 by 130% following a drought (Thom *et al*. 2013). In all cases the superior productivity and persistence of endophytes *per se* over endophyte-free and of AR37 itself have been attributed to the insect resistance of infected plants with differences exacerbated in dry conditions (Popay and Hume 2011). Differences in ryegrass productivity due to endophyte do not always occur. Despite tiller density differences, annual dry matter yields were similar in all treatments of the trial in the Waikato reported by Thom *et al*. (2013). Similarly in a 3-year experiment in Victoria, Australia, there were no differences in dry matter yield between AR1, AR37 and Wild-type treatments (Moate *et al*. 2012). During this trial, some paddocks had damaging populations of redheaded cockchafer (*Adoryphorus couloni)* and Tasmanian grass grub (*Aphodius tasmaniae*), neither of which was controlled by these endophytes.

Future prospects

Loline alkaloids have broad spectrum insect activity, including the potential to control root feeding grubs such as grass grub (*Costelytra zealandica*) (Patchett *et al*. 2011) in addition to other major insect pests already mentioned here. Moreover they have no known mammalian effects (Easton *et al.* 2009). They are produced by endophytes naturally infecting tall fescue (*Festuca arundinacea*) and meadow fescue (*F. pratensis*). These endophytes can form stable relationships with perennial ryegrass hosts but expression of the lolines and seed transmission of the endophyte is variable (Easton *et al*. 2009). Expression of lolines is influenced genetically by the host offering scope for selecting for increased concentrations. Overcoming these impediments will provide farmers with a safe and effective endophyte for insect control.

Conclusion

Fungal endophytes of ryegrass have been exploited to provide insect control with minimal effects on livestock health. Two such endophytes, AR1 and AR37, are delivering benefits in pasture productivity and persistence to New Zealand farmers.

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