Delivering endophyte benefits on-farm: pasture renewal success and effects on pest invertebrates

G.M. RENNIE, W.McG. KING, N.L. BELL and T.M. EDEN
AgResearch Limited, Ruakura Research Centre, Private Bag 3123, Hamilton 3240
grant.rennie@agresearch.co.nz

Abstract

New pasture grass-fungal endophyte associations have the potential to greatly improve the persistence of high performance pastures in New Zealand farm systems. However, there is increasing concern among farmers that the benefits of novel endophytes are not being delivered 'in-paddock'. This study aimed to determine the frequency of novel-endophyte infection in renovated paddocks and, further, the persistence of those grass/endophyte associations. In addition, the effect of endophyte on populations of pest invertebrates was investigated. Results showed endophyte infection levels in 80% of renovated pastures were sufficient (>70% tillers infected with endophyte) to provide the pest protection of which they are capable. The sown endophyte persisted in most pastures (74% average infection) with low standard endophyte (SE) contamination (5% average infection). Endophyte strain was shown to influence some pasture insect pest populations. Therefore, renewal of run-out pastures can successfully introduce new cultivars and endophytes into farm systems.

Keywords: Argentine stem weevil, AR1, AR37, black beetle, NEA2, pasture renewal, persistence

Introduction

The link between the presence of fungal endophyte (*Neotyphodium* spp.) in perennial ryegrass and ryegrass staggers in grazing animals and between endophyte and protection from insect herbivory has been known since the early 1980s (Fletcher & Harvey 1981, Prestige *et al.* 1982). Subsequent endophyte development work focused on substantially altering the alkaloid profiles so that few or no staggers symptoms were induced (Bluett *et al.* 2005). A strong uptake of these novel endophyte strains by New Zealand farmers has followed. For example, the AR1 endophyte represented around 80% of endophyte infected perennial ryegrass (*Lolium perenne*) seed sold in 2007 (Milne 2007).

The alkaloids produced by the endophyte confer a degree of protection from herbivory by a range of insect pests (Popay & Hume 2011, this volume). The standard (or 'wild-type') endophyte in New Zealand ryegrass pastures produces the alkaloids peramine, lolitrem B and ergovaline. The latter two alkaloids can cause a range of animal disorders such as ryegrass staggers and heat stress. The introduction of novel endophytes can increase animal production by avoiding the standard endophyte animal health problems, while providing some resistance to a range of insects. For example, AR1 endophyte produces high levels of peramine that affords protection from Argentine stem weevil (ASW) (*Listronotus bonariensis*); this endophyte also protects against pasture mealybug (*Balanococcus poae*). The NEA2 endophyte produces low levels of peramine and ergovaline and very low levels of Lolitrem B while the Endo5 endophyte produces ergovaline and peramine. These endophytes provide protection from African black beetle (*Heteronychus arator*) adults and have some activity against ASW. The AR37 endophyte produces a separate group of alkaloids, epoxy-janthitrems, and provides resistance to a broad range of insects including ASW, black beetle adults, pasture mealy bug and a root aphid (*Aploneura lentisci*) (Popay & Hume 2011, this volume). In tall fescue (*Festuca arundinacea; Schedonorus phoenix*) the Max P endophyte provides resistance to black beetle adults and ASW predation through production of peramine and loline (Popay *et al.* 2005).

Successful introduction of new endophyte pastures with persistence equal to or bettering standard endophyte pastures, has been demonstrated on dairy research farms in the Waikato (Thom *et al.* 2008; Bluett *et al.* 2005). However, there is increasing concern that the benefits shown on research farms are not being achieved by the general farming community.

This paper reports some results from 1 year of a multi-year trial to determine the drivers of pasture persistence and the value of pasture renovation to dairy farmers within the Waikato and Bay of Plenty (BOP) regions of New Zealand.

Methods

A total of 55 paddocks were selected from 10 farms in the Waikato and 10 farms in the BOP. Between two and four paddocks were chosen from each farm to represent various stages of pasture renewal. All newly renovated (<1 year old) and recently renovated (2–6 years old)

Figure 1 Endophyte infection frequency for all pastures sampled as determined by tissue print immunoblot assay.

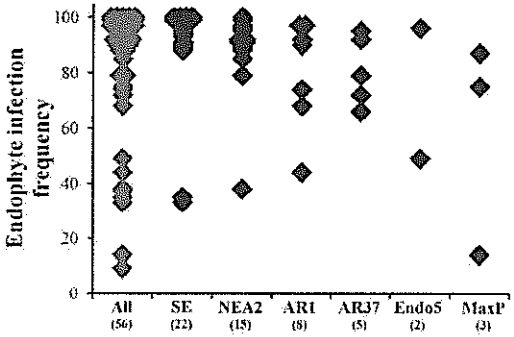
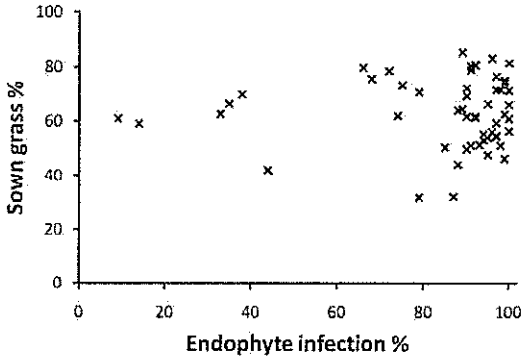


Figure 2 Relationship between the total endophyte infection frequency (novel endophyte and standard endophyte) and percentage of sown grass species in the total pasture sward.



pastures (33 in total) had been sown with ryegrass that contained a range of novel endophytes. These were compared with established (>6 years old) pastures with standard endophyte. Further description of the study has been published by Bell *et al.* (2009). Details of the pasture renovation, including cultivar and endophyte strain sown in each paddock, were determined through farmer interviews.

The endophyte frequency in the 55 pastures was determined by randomly sampling 100 tillers of the sown species (ryegrass or tall fescue) throughout the paddock, evenly spaced, on an 'N' shaped sampling transect. Sampling occurred during late spring (November 2009) to early summer (January 2010). Each tiller was cut to ground level and re-cut prior to examining for the presence of endophyte using a tissue-print immunoblot assay (Hahn *et al.* 2003). Where tiller blotting was delayed, tiller samples were stored under refrigeration (4°C) for no longer than 24 hours. Tiller samples were then frozen and stored for further analysis.

Endophyte strain in the paddocks sown with a novel endophyte was determined in 40 random tillers, of unknown infection status, selected from the tillers that had been frozen after having been blotted. Genetic identification of endophyte strain was performed using genomic DNA isolation with PCR using endophyte-specific simple sequence repeat (SSR) markers. SSRs used included B10 and B11 (Moon *et al.* 1999) and ans025, from a marker set described by Kirkby *et al.* (2011). AR37 endophyte culture isolate was used as a source of control DNA. Genotypic analysis and fragment sizing was conducted as in Kirkby *et al.* (2011).

A survey of the pasture pest invertebrate population was carried out in February 2010 with all pastures in the project sampled. In each paddock 10 spade squares (20 × 20 × 20 cm) along a central sampling transect through the paddock were manually dissected to collect the subterranean invertebrates present. The insect samples were preserved in ethanol for subsequent counting. Foliar dwelling insects were collected with a suction apparatus (modified garden blower/vacuum) (Phillips *et al.* 2000). A 30-m by 10-cm area was sampled by suction alongside the soil sampling transect. Each suction sample was stored in vented containers prior to separating and counting the insects present.

Results of individual insect paddock counts were analysed using a generalised linear model with Poisson error and log link. Due to unbalanced numbers of paddocks with different endophyte strains, the errors are reported as effective standard errors. To ensure that the specific endophyte was in a suitably high proportion of the pasture to influence the insect population, pastures with low infection levels (<70%) of the desired endophyte were excluded from the analysis. In total, 44 paddocks were used in the analysis. MaxP and Endo5 endophyte strains were excluded from the insect analysis due to a low number of eligible paddocks.

Results and Discussion

Endophyte infection

The endophyte frequency of the 55 pastures surveyed ranged from 9% to 100% with an average of 84% infection (median 92%) (Fig. 1). In novel endophyte pastures the endophyte frequency was high with an average of 80% of tillers containing endophyte (above 70% in 27 of 33 paddocks).

Of the seven pastures with infection rates of lower than 50%, one was sown with endophyte-free tall fescue (9% infection) one was standard endophyte pasture that had been oversown with endophyte-free annual ryegrass (35% overall infection) and five were sown with novel endophyte pastures. Five of these six pastures were classed by the farmer as "run out" regardless of age due to the invasion of weed species or poor pasture production caused by loss of ryegrass

Table 1 Infection rate of sown novel endophyte and standard endophyte contamination in renewed pastures. Endophyte type was determined by analysis of genetic markers.

	Mean infection rate (%)	Std. deviation	Range
Novel endophyte	74	24	0 to 95
Standard endophyte	5	6	0 to 18
Nil endophyte/no result	21	23	0 to 93

plants. The remaining pasture had been recently sown (pasture age <6 months) and was only entering its first summer. Discussion with the farmer on his pasture renewal procedures showed that the seed was sown early during a dry period and an extended time to germination may have contributed to a loss of endophyte viability.

The SSR analysis of endophyte strain showed that the infection level of the strain sown in all renovated pastures (<6 years) was above 70% in most pastures (25 of 32) (Table 1). The average level of standard endophyte contamination in novel endophyte pasture was low with most samples (25 of 32) at or below 10% standard endophyte infection.

The high proportion of novel-endophyte tillers and the low level of contamination with standard endophyte showed that little reversion to previously established pasture has occurred. There was no difference in novel endophyte infection level or standard endophyte contamination between endophyte strains. Standard endophyte contamination did not significantly increase from the newly renovated pasture (<1 year) to the recently renovated pastures (2 to 6 years). However, two pastures sown with novel endophyte infected grass had no novel endophyte present in the tillers analysed. Both of these recently renovated pastures (5 and 2 years since renovation) were deemed to be 'run-out' by the farmers due to poor pasture persistence, due probably to poor survival of endophyte-free plants, and had low standard endophyte contamination (8 and 10% respectively). The lack of novel endophyte survival in plants is likely caused by sowing seed without viable endophyte. While the endophyte viability in quality seed sold in NZ is high at the time of certification (industry standard of >70%), various factors prior to sowing, such as temperature or length of storage, may reduce viability (Welty *et al.* 1987).

A low endophyte percentage in an established sward would be expected to have a detrimental effect to the persistence of the pasture. Pasture pest insect feeding on the endophyte-free portion of the ryegrass population can result in a rapid loss of grass plants and an opening of the pasture sward (Popay & Hume 2011, this volume). However, in this study, there was no relationship between total infection frequency of all endophytes present in pasture and the average proportion of the sward that the sown grass represented from November 2009 to November 2010 (Fig. 2).

Invertebrate sampling

The total foliar-dwelling insect population was influenced by the endophyte strain present in the sown pasture species. The population of foliar insects was significantly higher ($P<0.05$) in pastures sown with the NEA2 endophyte (Table 2), mostly due to significantly higher populations of ASW. The NEA2 endophyte has been shown to produce levels of peramine insufficient to control ASW (Popay & Hume 2011, this volume). There was no significant difference between ASW populations under AR1, AR37 and standard endophyte. There was no significant difference between populations of non-ASW foliar insects (data not shown).

The total soil-dwelling, insect population was also affected by endophyte with a significant ($P<0.05$) reduction in insects in soil beneath pastures sown with NEA2 compared with standard endophyte (Table 2). Total soil-dwelling insects include non-grass and non-plant feeding insects as well as predators. Not all insects would therefore be directly influenced by endophyte presence in grasses. Although white fringed weevil (*Naupactus leucoloma*) prefer white clover as a host (Hardwick *et al.* 1999) fewer were found under NEA2 than under standard endophyte pastures, although the observed populations under all endophytes were below damaging thresholds (King *et al.* 1982). There was no significant difference in black beetle populations under the four endophyte treatments.

Table 2 Mean insect populations (effective standard errors in parentheses) in pastures of different endophyte strain (SE = standard endophyte, n = number of paddocks). Significant differences ($P<0.05$) are indicated by differing letters following means.

	Foliage insects /m²		Soil insects /m²		
Endophyte	Total	Argentine stem weevil	Total	Black beetle	White fringed weevil
SE (n=20)	25a (4)	15a (3)	103a (15)	35 (6)	40a (4)
NEA2 (n=14)	49b (6)	32b (4)	57b (14)	27 (7)	2b (6)
AR1 (n=6)	22a (6)	10a (4)	120ab (38)	49 (18)	35ab (6)
AR37 (n=4)	15a (5)	7a (3)	96ab (30)	29 (11)	15ab (5)

The AR37 and NEA2 endophytes have been selected to provide protection against adult black beetle. The level of alkaloids in the endophytes, janthitrems in AR37 and ergovaline in both NEA2 and the standard endophyte, have contributed to keeping the populations below damaging levels (taken to be <40 m^2, Watson *et al.* 1980). In contrast, AR1 endophyte is known to have only limited black beetle resistance (Popay *et al.* 1999). However, black beetle numbers were not significantly different between endophyte strains, in part due to a low number of samples and large variability in black beetle numbers. The majority of the black beetle encountered during sampling were larvae (79%) which are not directly affected by endophyte in the plants because alkaloids are not present in the plant roots to any great degree. Endophyte does, however, have an effect on feeding and subsequent egg-laying of the adults, depending on the strain. Black beetle have reached damaging populations in many parts of the Waikato and BOP regions, including 18 of the trial sites in the last 2 years (Bell *et al.* 2009; pers obs).

Most pastures over 2 years of age had a non-sown component of annual grasses (data not shown) that can provide a food source for pest insects. During the period of endophyte and insect sampling (November through February) the sown pasture species (ryegrass or tall fescue), on average, formed 82% of the total grass component. The remainder was made up of annual weed grasses or unsown perennials (unpublished data).

Conclusion

The introduction of new grass endophytes into New Zealand farm systems is commonly being achieved by farmers. Through effective, best-practice pasture renewal it can be expected that the potential persistence benefits of pastures with novel endophyte can be achieved. Unequivocal evidence of the benefits of novel endophytes to pasture persistence will require longer-term study.

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