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The role of perennial ryegrass endophyte in Italian ryegrass

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Abstract. Italian ryegrass (*Lolium multiflorum*) and short-term hybrid ryegrass (*L. boucheanum*) have reliable establishment and high cool season growth, but varying persistence after the first summer. In Northland, there is increased stress on ryegrass from invertebrate insect attack. Selected strains of endophyte (*Neotyphodium lolii*) have been identified that protect perennial ryegrass from invertebrate attack. Two of these endophyte strains (AR1 and AR37) were inoculated into two Italian ryegrass cultivars (Status and Corvette) and compared in a small plot agronomic trial with the same cultivars free or low in the naturally-occurring endophyte *N. occultans*. From April 2004 to June 2005, ryegrass plots with these endophyte-cultivar combinations were measured for dry matter yields and plant survival under summer/autumn insect pressure in Northland. In autumn/winter 2005, novel endophytes resulted in greater plant survival and yield advantages (82%) than the same cultivars with no/low *N. occultans* endophyte. These effects were consistent across cultivars. Increased agronomic performance corresponded with lower damage from African black beetle larvae (*Heteronychus arator*). This result has implications for extending the persistence and potential yields of Italian/hybrid ryegrass pastures subject to pest attack.

Introduction

Italian and short-term hybrid ryegrasses (*Lolium multiflorum* and *L. boucheanum* syn. *L. hybridum*) are increasingly being used by farmers in Australia and New Zealand (NZ). Use of these ryegrasses increased from 30% of seed sales in the Waikato and 50% in the Northland regions of NZ in 1990, to 40% in Waikato and 60% in Northland in 2005 (Anon. seed company).

Much of their usage is as a renovation tool, in overdrilling runout pastures in autumn before returning to permanent pastures. Their vigorous establishment and early growth suppresses pasture weeds and may reduce populations of pasture pests particularly those affecting legumes. The other key feature of these ryegrasses is their superior autumn to early spring growth potential compared to that of perennial ryegrass (*L. perenne*) (Lancashire 1982). In particular in Northland, Italian/hybrid ryegrasses are overdrilled in autumn into Kikuyu grass (*Pennisetum clandestinum*) pastures to provide winter-spring production, a period when Kikuyu growth rates are very low.

Pastures in northern NZ are subject to variable summer rainfall and considerable damage from pasture pests. Under these conditions, survival of Italian/hybrid ryegrasses is short-lived and typically only 9 months. In both NZ and Australia, persistence and production of perennial ryegrass and tall fescue (*Schedonorus phoenix* syn. *Festuca arundinacea*) can be improved by infection with a symbiotic fungal endophyte (*Neotyphodium lolii* and *N. coenophalium*, respectively), compared with the same cultivars free of endophyte (Cooper *et al.* 2002, Popay *et al.* 1999, Wheatley *et al*. 2003). This can be attributed to increased resistance to insect pests and in tall fescue a greater tolerance to soil water deficits.

In NZ, Italian/hybrid ryegrasses are frequently infected with the *N. occultans* endophyte (Forde *et al.* 1998, Moon *et al*. 2000). This endophyte imparts only limited advantages to these plants possibly only at the seedling stage through protection from insect attack (see Hume *et al*. 2001). Italian/hybrid ryegrasses are occasionally found to be naturally infected with *N. lolii*, which provides the plant with improved resistance to the insect pest Argentine stem weevil (*Listronotus bonoriensis*) (Piggot et al. 1988, Hume *et al*. 2001).

Selected endophytes from perennial ryegrass have been successfully inoculated into Italian/hybrid ryegrasses. This paper reports an agronomic trial sown in Northland to determine the effects of these endophytes on the production and persistence of Italian/hybrid ryegrasses.

Materials and methods

A small plot agronomic evaluation was sown at Kerikeri, Northland to compare "Grasslands Status" and "Corvette" Italian ryegrasses infected with *N. lolii* endophytes derived from perennial ryegrass (AR1 or AR37) and the same cultivars free of or with low infection of *N. occultans* ('nil/low' endophyte treatment). *N. occultans* is the endophyte that naturally infects these ryegrasses. These six treatments were sown as plots of 2.5 x 3 m with four replicates in a randomised block design, along with 13 other cultivars/selections of Italian ryegrass.

The trial was sown in autumn 2004 (8 April) into a finely cultivated Okaihau gravely clay, a free draining volcanic soil subject to African black beetle (*Heteronychus arator*) damage over summer/autumn. Seed was sown at 20 kg/ha and herbicides were used to control other species. Herbage dry matter yields were measured by cutting a mower strip to 3 cms height from the length of each plot when growth attained 25-30 cm height. These growth cuts were undertaken on 10 occasions between June 2004 and August 2005. After each cut, plots were grazed by mature sheep and uneven residuals trimmed by rotary mower as required. Nitrogen was applied at a rate considered to be 'non-limiting' of 92 kg N/ha (200 kg urea/ha) after each defoliation.

The occurrence of endophyte in 40 individual ryegrass tillers per plot was determined at 7 and 10 months (late spring and late summer, respectively) after sowing. This was done using a tissue print immuno-blot assay (Hahn *et al.* 2003).

In late summer, African black beetle larvae were detected throughout the trial resulting in wilting of plants and dead patches in the worst affected plots. These signs of larval damage were visually scored by assessing plots on 21 Feburary 2005 (0 = no damage, 5 = severe damage). Transect lines set diagonally across each plot were assessed for the occurrence of ryegrass plants in mid-autumn 2005 (19 April) (100 points per plot).

Results and Discussion

Testing ryegrass tillers for endophyte presence showed that the sown plots had similar % endophyte to that of the sown seed. AR1 and AR37 infected plots had over 80% infected tillers (mean 89%) for both cultivars. Nil/low plots had 15% *N. occultans* for Status and 0% for Corvette. Percentage infection did not differ between the two sampling dates.

Both cultivars performed similarly and there were no significant interactions of cultivar with endophyte. The results for both cultivars have therefore been combined. Ryegrass dry matter yields were similar for all endophyte treatments (Fig. 1) from establishment until mid-summer ($P > 0.05$). From late summer of 2005 onwards significant differences occurred between endophyte treatments with yields lowest in Nil/low plots, greatest in AR37 plots and intermediate in AR1 plots (Fig. 1 and Table 1) ($P < 0.001$). This was most likely due to the differences in plant frequency among these treatments in early autumn 2005 (Table 1) ($P > 0.001$), a result of the late summer damage to plots caused by the root feeding of black beetle larvae (Table 1) although this damage score did not reach statistical significance ($P > 0.05$).

Better performance of these Italian ryegrasses when infected with endophytes from perennial ryegrass (*N. lolii*) is consistent with the results reported by Piggot *et al*. (1988) and Popay *et al*. (1995). This result is similar to the better protection that AR1 and AR37 afford perennial ryegrass in this environment through improved pest protection compared with no endophyte (Popay *et al.* 1999, Hume *et al.* 2004). It shows the potential in Northland for these endophytes to extend persistence of these ryegrass types into a second year, obviating (or removing) the need to resow or allow natural reseeding to occur.

A similar trial was sown in autumn 2004 at Palmerston North, Manawatu, a location that is not subject to the insect pressure and the severity of summer-autumn soil moisture deficits as the Kerikeri site. At this Palmerston North site there were no significant effects of endophyte in Status or Corvette on ryegrass yields (TB Lyons unpublished data) ($P > 0.05$). The use of AR1 or AR37 endophytes in Italian/hybrid ryegrasses may therefore be of greatest value in extending grass longevity in those regions subject to insect pressure such as black beetle and/or Argentine stem weevil.

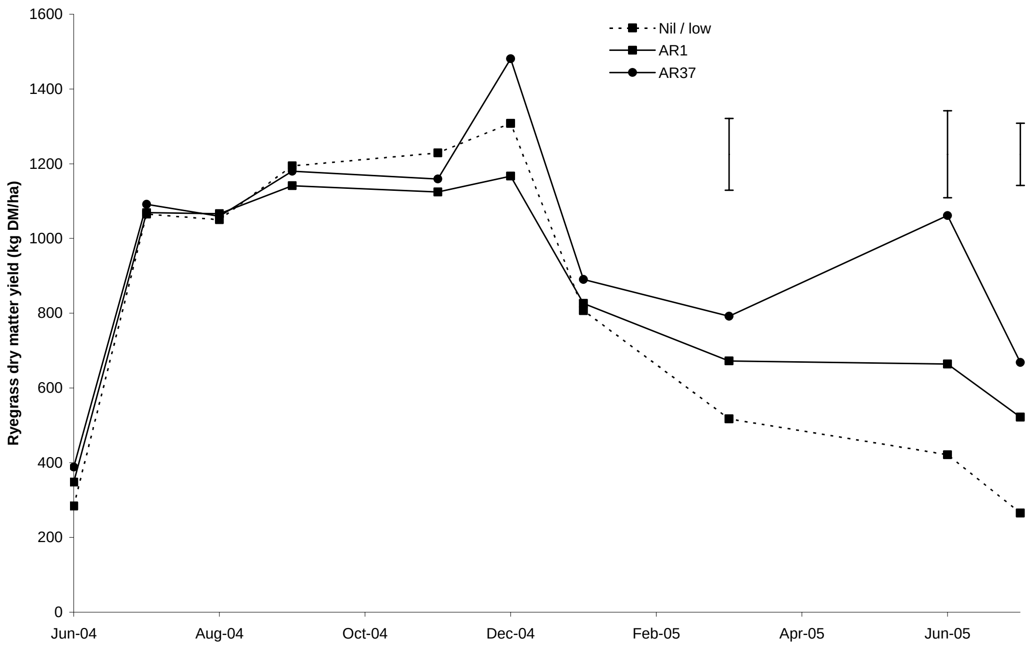

Fig. 1. Harvested ryegrass dry matter yields for 3 endophyte treatments at Kerikeri, Northland (mean of cultivars). Error bars show l.s.d. (=0.05) values for harvests when significant differences occurred.

Table 1. Effect of endophyte on mean damage score due to black beetle larvae feeding in late summer 2005, ryegrass plant frequency per transect (n=100 points) in mid-autumn 2005, and ryegrass dry matter yields (kg DM/ha) in autumn/winter 2005. Mean of all cultivars

Endophyte	Black beetle damage score	Ryegrass plant frequency	Ryegrass yield
Nil/low	2.8	16 c	1200 c
AR1	1.4	23 b	1860 b
AR37	1.1	30 a	2520 a
P value	$P > 0.05$	$P < 0.001$	$P < 0.001$
l.s.d. (= 0.05)	1.8	5	127

Values within a column with the same letter are not significantly different at $P = 0.05$.

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