Improvement in the nitrogen use efficiency and uptake of perennial ryegrass (*Lolium perenne)* caused by the *Epichloë* fungal endophyte AR37

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Abstract

The forage yield and pasture persistence benefits of *Epichloë* fungal endophyte strain AR37 (AR37) in high rainfall perennial ryegrass (*Lolium perenne*) pastures in Australia and New Zealand are recognised. However, the effect of beneficial endophytes on nitrogen (N) use efficiency, yield response to applied N and N uptake had not been determined.

To investigate this, a replicated trial was managed for three years under irrigation in southern Australia. Five N application rates (0, 20, 40, 80 and 160 kg N/ha) were applied every second harvest to a tetraploid perennial ryegrass variety with and without AR37. Ryegrass biomass was determined by full plot harvest and N percentage determined by near infrared spectroscopy.

AR37 caused an increase in perennial ryegrass N use efficiency (the slope of the N response function) in all seasons, with the average increase in marginal response being 2.1 kg DM/kg N or 29.6 % over three-years. Benefit at individual harvests ranged from 0.2 to 6.5 kg DM/kg N. Foliage N percentage was not influenced by AR37 infection, thus AR37 caused enhanced N uptake due to increased forage yield.

This trial demonstrates AR37 can contribute to the profitable and sustainable intensification of high rainfall perennial ryegrass pastures by significantly increasing the marginal yield response to N, and the uptake of applied N fertiliser. Further, increased N uptake caused by AR37 will likely have implication for negative environmental externalities associated with N use (leaching, runoff and nitrogenous greenhouse gas emissions) which warrants further investigation, along with research to clarify biotic and abiotic drivers of observed results.

Introduction

Novel fungal endophytes (*Epichloë festucae* var. *lolii,* formally *Neotyphodium lolii*) of perennial ryegrass such as the strain AR37 were intentionally discovered and commercialised to address both the animal health effects associated with wild type endophytes (Caradus et al. 2020) and pasture persistence problems associated with a plant either not hosting an endophyte (nil endophyte) or hosting an endophyte ineffective against insect pests. Animal productivity benefits of novel endophytes as compared to their toxic wildtype counterparts are thoroughly reviewed by Caradus et al. 2020 and include improved milk and meat production; while enhanced animal welfare is realised via a reduction in heat stress and ryegrass staggers (Fletcher et al. 2017). Improved resistance to insect pests also causes benefit via increased pasture persistence and forage yield (Popay and Hume 2011). Novel endophytes are now widely marketed and cultivars containing them are Australia and New Zealand's highest yielding perennial ryegrass cultivars as determined by their respective independent forage value indexes. Higher rainfall zones of south eastern Australia host a range of pasture insect pests (Umina et al. 2021), and consequently a fungal endophyte providing consequential resistance to these (an *effective endophyte*) is critical for perennial ryegrass agronomic performance in these agroclimatic areas.

While the pasture persistence and forage yield benefits of effective endophytes have been demonstrated, the effect of endophytes on pasture response to applied N have not, and this may be of consequence. Economically the response of a pasture to applied N governs a farmer's financial return from the management practice; and via the marginal cost principal, the shape of the response function governs how much a farmer should rationally apply. From an environmental perspective the more N used for growth, the less that is available to cause negative externalities such as N leaching, runoff or nitrification (Moir et al. 2012).

In this study it was hypothesised infection with an novel effective endophyte would alter perennial ryegrass response to applied N. A four year field study was completed to address the hypothesis and here an initial summary of our results is presented. An 'effective endophyte' is defined as one capable of providing significant agronomic benefit in a given environment, and not all endophytes can achieve this.

Methods

The four year trial (2014-2017) was sown in autumn 2014 into a prepared seed bed at DLF Seeds' Research Farm in Ballarat, Victoria, Australia. The soil at this site is a deep red Krasnozem, weathered *in-situ* from basalt. Seed bed preparation commenced the year prior with an autumn sown green manure lupin (*Lupinus angustifolius*) crop terminated with glyphosate in the spring prior, and the site being chemically fallowed over the summer of 2013/14. Soil tests (0-10 cm) at sowing demonstrated non-limiting fertility: pH (H_20) 5.3; electrical conductivity (saturated extract) 1.0 dS/m; Olsen P 37 mg/kg; Colwell K 320 mg/kg; and sulphate sulfur (KCl40) 7 mg/kg. To ensure the trial remained unconfounded by the concentration of these nutrients, twice yearly basal application of P, K and S occurred.

The trial utilised tetraploid perennial ryegrass cultivar (cv.) Base, commercially marketed in combination with the AR37 endophyte. A control (endophyte negative) treatment of cv. Base was generated by incubation of the seed at elevated temperature and humidity. Post establishment immunoblot assays confirmed successful elimination of the AR37 endophyte from the control (endophyte negative) line, while Base AR37 was confirmed to have ~ 94 % of plants infected with the novel endophyte. Cultivar Base is of north west Spanish genetic origin, accordingly good autumn and winter growth was expected in the test location where daily maximum and minimum temperatures through winter are approximately 10 ºC and 3 ºC respectively.

The three rep trial was laid out in an RCB design with 8 columns and 18 rows with each variety/fertiliser treatment receiving 0, 20, 40, 80 or 160 kg N/ha as urea after every even numbered harvest. Harvested plot area was 0.8 x 3.2 m. The trial contained other variety and fertiliser treatments not reported here.

Harvest occurred when ryegrass reached the three leaf stage as is best agronomic practice. For dry matter and N percentage determination, pre-harvest approximately 300 g of forage was sub-sampled from each plot 50 mm above ground level. Plots were harvested using a commercial mower modified with load cells to measure the weight of fresh forage removed. Twenty two harvests occurred throughout the trial duration.

Irrigation was applied via a travelling boom as required through summer to allow growth, although breakdowns in the summer of 2016/17 caused growth then to be constrained by low soil moisture availability.

Analysis utilised a linear mixed model considering row, column, rep, linear row and linear column effects, with spatial variation further considered by the approach of Cullis and Gleeson (1991). Empirical Best Linear Unbiased Estimators (EBLUEs) were generated for each harvest utilising a model optimised to the harvest. Despite N input occurring after harvest two and four, no forage yield response to N was observed until harvest 5 on 26 February 2015. This is likely due to a large supply of mineralised N which accumulated prior to and post sowing. Each subsequent harvest displayed an N response with exception of harvest 17 when a lack of irrigation severely constrained pasture growth. To investigate AR37 endophyte's seasonal effect on N response, harvests were grouped into seasons utilising harvest date and correlation between EBLUEs at each cut date. Seasonal EBLUEs were then generated utilising three summer, six autumn, three winter and four spring harvests.

Results and Discussion

While forage yield response to fertiliser is broadly characterised by diminishing returns relationships, none were consistently identified within the range of N application rates used in this experiment. This was evidenced by the good to excellent linear correlations observed between N application rate and yield both at each harvest where response to N occurred, and in each of the aggregated seasonal analysis. If N treatments greater than 160 kg N/ha had occurred, it is possible diminishing returns would have been observed. As linear correlations fit, the 'rate of response' (kg DM/kg N) referred to from here on is the slope of fitted linear equations, i.e. on 28 March 2017 the rate of response of Base AR37 to N was 13.09 kg DM/kg N as the yield response of Base AR37 to N was described ($R^2 = 0.99$) as *yield = 13.09 * N rate + 1,333*.

Impact of AR37 endophyte on N use efficiency over time

To consider the effect of AR37 over time, differences in the rate of response of the AR37 and nil endophyte treatment are compared in Figure 1.

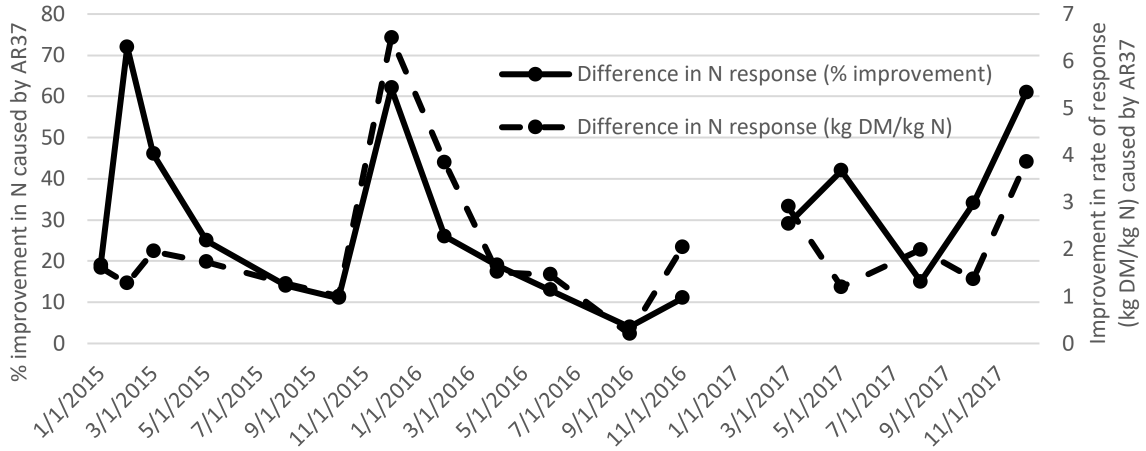

Figure 1. Absolute (dashed line) and percent (solid line) improvement in the N response of AR37 as compared to nil endophyte ryegrass over time.

Infection with AR37 had a positive effect on ryegrass yield response to N ranging an additional 0.20 to 6.50 kg DM/kg N. A seasonal pattern is evident in AR37's benefit. In summer and autumn each year AR37 inferred a greater increase in response to N, ranging 26 (autumn 2016) to 72 % (autumn 2015) more yield per unit of applied N than the nil endophyte control. AR37's N response benefit waned each winter through early spring, however remained in the order of 10 to 20 % with exception of the 19 September 2017 harvest.

Seasonal impact of AR37 endophyte on N use efficiency

As observed N response benefits varied seasonally, seasonal data were aggregated.

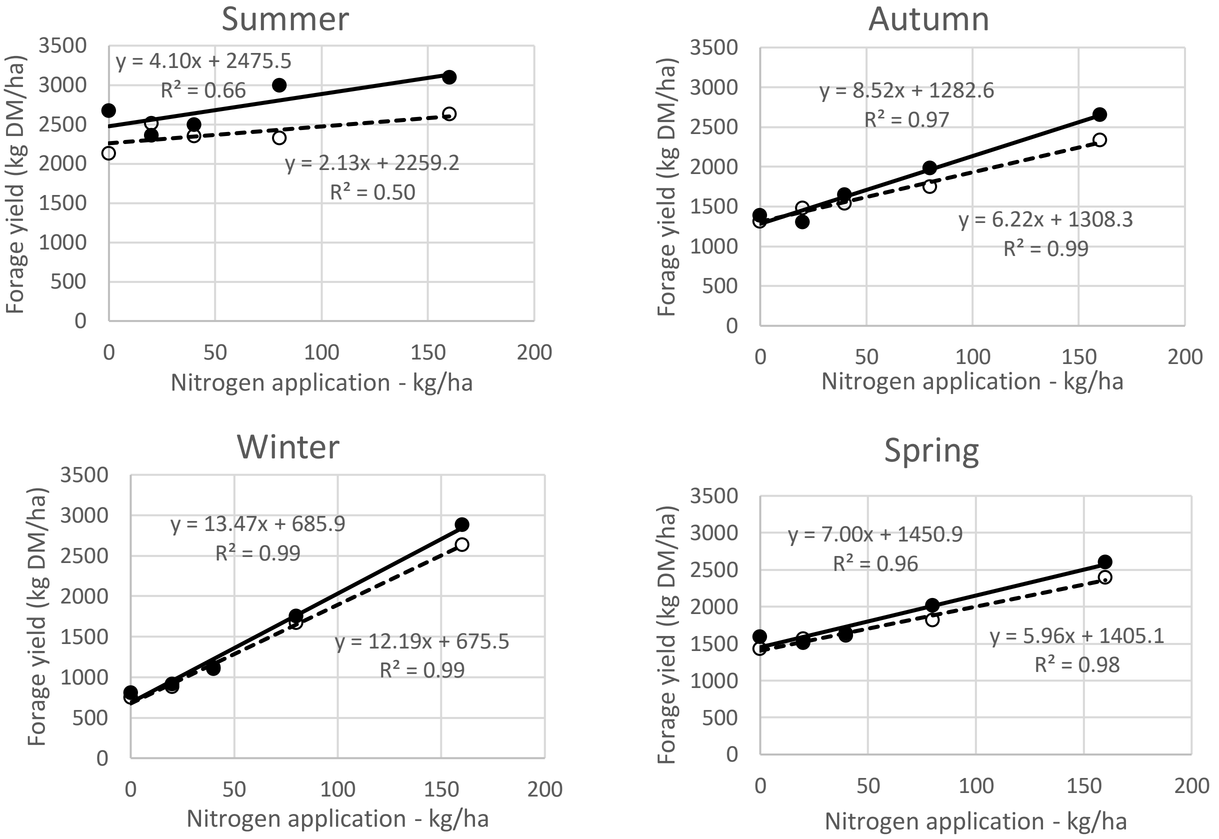

Figure 2. Seasonalised N responses for AR37 (closer circles and solid line) and nil (open circles and dashed line) ryegrass.

Ryegrass with the AR37 endophyte had improved response to N fertiliser as compared to the nil endophyte ryegrass in summer (1.96 kg DM/kg N improvement), autumn (2.30 kg DM/kg N improvement), winter (1.28 kg DM/kg N improvement) and spring (1.04 kg DM/kg N improvement).

AR37 impact on seasonal N uptake

While N % of perennial ryegrass dry matter did increase with increasing N fertilisation, the rate at which it did so was almost unaffected by AR37, with the AR37 and nil lines having essentially identical N % in any given season and at any given N fertiliser rate (data not shown). Accordingly, AR37 was responsible for an increase in the amount of applied N utilised by ryegrass in all seasons, but this occurred via an increase in yield rather than N %.

Conclusions and Implications

Ryegrass infection with an effective endophyte such as AR37 appears critical to maximise the response of perennial ryegrass to applied nitrogen fertiliser at this trial site. This study did not investigate the causal mechanism of the AR37 induced improvement, however it is suspected to be resistance inferred to the root aphid *Aploneura lentisci*, as it is frequently identified at the trial site during summer and autumn (Popay et al. 2021) on roots of nil endophyte plants, but not on the roots of AR37 plants. As this root aphid is widely encountered in perennial ryegrass pastures in Australia (Umina et al. 2021) it may be that an effective endophyte is necessary to maximise the efficiency with which N fertiliser is utilised in many Australian ryegrass pastures.

The economic benefits of effective endophytes such as AR37 appear far from trivial. Even assuming conservative N application rates and forage values, via improved resource use efficiency, they will likely contribute hundreds of dollars per hectare per year of value to producers. In addition, rather unintuitively, should the increase in the slope of the N response curve caused by AR37 hold at application rates in the range where the response curve asymptotes, this will in fact increase the amount of N fertiliser a rational profit maximising producer should choose to apply, further compounding the positive economic benefits of effective endophytes.

From an environmental perspective, effective endophytes will likely have positive impact on N leaching as this inversely correlates with pasture N uptake. Further, endophyte enhanced uptake of N may have implications for atmospheric losses via nitrification, a worthy area for future research.

European legislators may wish to reconsider their now outdated and unnecessary (in light of animal safe endophytes) restrictions on the use of endophytes should these benefits be demonstrated in European farming systems.

This study demonstrates effective endophytes like AR37 can contribute to both profit maximisation from N fertiliser use and the sustainable intensification of ryegrass farming systems. Further analysis and modelling of available data will occur to better understand the production, economic and environmental implications identified.

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References

Caradus, J.R., Card, S.D., Finch, S.F., Hume, D.E.., Johnson, L.J., Mace, W.J. and Popay, A.J. 2020. Ergot alkaloids in New Zealand pastures and their impact. *New Zealand Journal of Agricultural Research*., July 2020.

Cullis, B.R. and Gleeson A.C. 1991. Spatial analysis of field experiments-an extension to two dimensions. *Biometrics*., 47(4): 1449-60.

Fletcher, L.R., Finch, S.C., Sutherland, B.L., deNicolo, G., Mace, W.J., Koten, C. and Hume, D.E., 2017. The occurrence of ryegrass staggers and heat stress in sheep grazing ryegrass-endophyte associations with diverse alkaloid profiles. *New Zealand Veterinary Journal*., 65(5): 232-241.

Moir, J.L., Edwards, G.R. and Berry, L.N. 2012. Nitrogen uptake and leaching loss of thirteen temperate grass species under high N loading. *Grass and Forage Science*., 68(2): 313-325.

Popay, A.J. and Hume D.E. 2011. Endophytes improve ryegrass persistence by controlling insects. *Pasture Persistence – Grasslands Research and Practice Series 15*: pp149-156

Popay, A.J., Hume, D.E., Mace, W.J., Faville, M.J., Finch, S.C. and Cave V. 2021. A root aphid *Aploneura lentisci* is affected by *Epichloë* endophyte strain and impacts perennial ryegrass growth in the field. *Crop and Pasture Science*., 72(2): 155-164.

Umina, P.A., Kemp, S., Babineau, M., Maino, J.L., Roberts, I., Govender, A., McDonald, G., Popay, A.J., Hume, D.E., Hardwick, S., Richards, N.K., Reynolds, O. and Chirgwon, E. 2021. Pests of Australian dairy pastures: distribution, seasonality and potential impacts of pasture production. *Austral Entomology*., 60(4): 763-781.