

## Management of novel-endophyte ryegrass pastures

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### Introduction

This paper discusses how to establish and manage pastures containing perennial ryegrass infected with a novel, or new, endophyte. With advanced planning, contamination with the naturally occurring wild-endophyte-infected ryegrass can be minimised so farmers obtain the benefits offered by novel endophytes, such as no ryegrass staggers.

### What is an endophyte?

An endophyte is a fungus that grows inside a host plant and protects it from insect attack. Most perennial ryegrass (*Lolium perenne*) plants in Victoria are infected with a naturally occurring wild endophyte (*Neotyphodium lolii*) (Reed *et al.*, 2000). This endophyte can only be seen under a microscope. Perennial ryegrass infected with endophyte produces chemical compounds called alkaloids that vary in concentration over the year. Some alkaloids enhance the persistence and productivity of ryegrass pastures in New Zealand and Australia by protecting them against insects. However, other alkaloids cause poor animal performance and health when consumed at too high a concentration. These include lolitrem B, which causes ryegrass staggers, and ergovaline, which can worsen heat stress.

### What is a novel endophyte?

Novel, or new, endophytes have been selected to avoid the animal health and production problems associated with perennial ryegrass infected with the wild endophyte, while maintaining positive effects on plant persistence. AR1 is an example of a novel endophyte, which has been selected by AgResearch in New Zealand because it does not produce lolitrem B or ergovaline but still produces the alkaloid that reduces insect attack, namely, peramine. AR1 was released in a number of commercial perennial ryegrass cultivars in New Zealand in autumn 2001. Grazing trials have shown that perennial ryegrass infected with AR1 endophyte can improve liveweight gain in sheep (Fletcher, 1999) and milk production in dairy cows (Bluett, unpublished data) when compared to the same perennial ryegrass cultivar containing wild endophyte.

### What is contamination?

Contamination refers to establishment of wild-endophyte-infected perennial ryegrass plants in an endophyte-free or novel-endophyte-infected pasture. Wild endophyte and novel endophytes look the same under the microscope, but since AR1 endophyte does not produce lolitrem B, showing the presence of lolitrem B by laboratory testing indicates that the

pasture has been contaminated with wild-endophyte-infected ryegrass. Contamination needs to be prevented to ensure that farmers gain the advantages of using a pasture containing a novel endophyte, such as no ryegrass staggers. When establishing and maintaining a novel-endophyte pasture, the main sources of contamination are from surviving vegetative ryegrass plants; seed on the soil surface after natural reseeding; seed buried in soil; and seed transferred into pasture in hay, machinery, and dung.

### How does endophyte spread?

When perennial ryegrass is leafy, the endophyte is concentrated in the leaf sheath, which is at the base of the plant. As perennial ryegrass becomes reproductive from late spring to late summer, the endophyte grows up the elongating stem and into the developing seeds. Mature seed is shed onto the soil surface by the process of natural reseeding (Figure 1). The seed containing the endophyte then germinates and establishes in the pasture. The endophyte *cannot* be spread from one plant to another.



**Figure 1: Ryegrass seed containing wild endophyte on the soil surface after making hay. This seed could contaminate a newly sown novel-endophyte-infected ryegrass pasture.**

### How long does ryegrass seed survive in the soil?

Most perennial ryegrass seed arising from natural reseeding over summer will germinate in the autumn, depending on rainfall. Some seed may become buried by livestock treading, earthworm and insect activity, and water or wind movement into soil cracks (Hume, 1999). Perennial ryegrass seed has limited dormancy and therefore relatively short survival in soil. However, work in New Zealand has shown that ryegrass seed and its endophyte can survive up to 2 years in some soils and climates.

### How to prevent contamination

This is best done by preventing natural reseeding, killing all existing ryegrass plants, depleting levels of buried ryegrass seed through cultivation or a fallow period, providing a seedbed that favours the rapid establishment of the sown ryegrass, and preventing seed dispersal in dung and hay and on machinery. Best results will be achieved through advanced planning. Both management of the existing pasture before renewal and the establishment method used for the new pasture can influence the amount of contamination that occurs.

### Pre-sowing pasture management

Management of the existing wild-endophyte-infected pasture the summer before sowing the novel-endophyte-infected pasture can control the amount of natural reseeding. Spraying of perennial ryegrass pasture and sowing a summer crop (turnips, maize, etc.) can eliminate reseeding. Cropping is therefore recommended before sowing a novel-endophyte pasture. However, if going from grass to grass, do not make hay off the paddock in the summer before renewal. This is because large amounts of endophyte-infected perennial ryegrass seed will be shed onto the soil surface, increasing the chances of contamination.

An experiment was carried out at Dexcel in Hamilton, New Zealand, from September 1999 to March 2002 to show how pre-sowing pasture management and establishment method influenced the contamination of a newly sown AR1-endophyte-infected ryegrass dairy pasture by wild-endophyte-infected ryegrass (see Bluett *et al.*, 2001 for more details). Pre-sowing management treatments were: (1) hay making, (2) silage making, (3) grazing by dairy cows, (4) grazing by dairy cows followed by topping, and (5) turnip crop. These treatments produced large differences in the density of viable ryegrass seeds on the soil surface in early autumn (Figure 2). These results clearly show that the amount of natural reseeding is high after making hay and low after growing turnips.

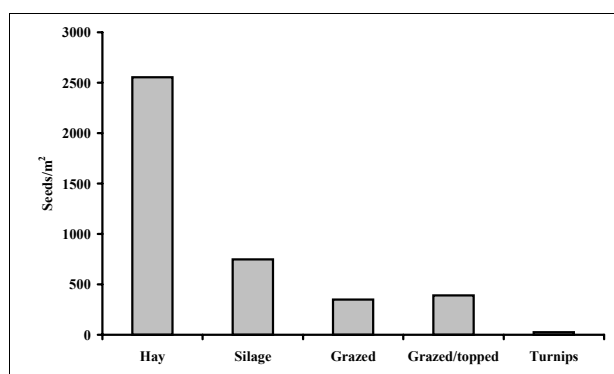


Figure 2: The effect of different pre-sowing managements on the viable ryegrass seed density (seeds/m<sup>2</sup>) on the soil surface in autumn 2000.

### Establishment method

The aims of the establishment methods are to eliminate all existing ryegrass plants, reduce ryegrass seed levels on the soil surface, and provide a seedbed that favours the rapid establishment of the sown ryegrass.

Following the above pre-sowing management treatments, the experiment compared the following establishment methods: (1) spray/cultivation, (2) spray/fallow/spray, and (3) hard-grazing (see Bluett *et al.*, 2001 for more details). AR1-endophyte-infected perennial ryegrass was then direct drilled into all treatments in autumn 2000. Contamination was assessed by measuring the concentration of lolitrem B in bulked ryegrass samples (Table 1) and determining the proportion of endophyte-infected perennial ryegrass tillers not producing lolitrem B (Table 2).

Table 1: The effect of establishment method on lolitrem B concentration ( $\mu\text{g/g}$ ) in bulked ryegrass samples collected over 2 years from direct drilled AR1-endophyte-infected ryegrass.

Establishment method	14 March 2001	11 March 2002
Spray/cultivation	0.3	0.1
Spray/fallow/spray	0.5	0.2
Hard-grazed	1.1	1.0
Nearby existing pasture	2.0	1.5

Table 2: The effect of pre-sowing management and establishment method on the percentage of endophyte-infected perennial ryegrass tillers infected with AR1 on 1 February 2001 (1 year after direct drilling).

Pre-sowing management	Establishment method		
	Spray/cultivation	Spray/fallow/spray	Hard-grazed
Hay	99	90	17
Silage	100	97	37
Grazed	100	99	57
Grazed/topped	100	99	52
Turnips	100	99	93

The spray/cultivation method resulted in the lowest levels of contamination (Tables 1 and 2) by killing existing plants using herbicide and then burying surface seeds by ploughing. The spray/fallow/spray method also had low contamination levels (Tables 1 and 2) by killing existing plants using herbicide, then allowing surface seeds to germinate after rain during the fallow period before respraying. A double rather than a single spray will greatly reduce contamination (Burggraaf and Thom, 2000). The timing of the second spray is critical and must occur after seedling growth, but there is a risk that more seedlings could germinate after the second spray.

As expected, direct drilling AR1-endophyte-infected seed into hard-grazed pasture resulted in a poor establishment of sown ryegrass and a high level of contamination because of the presence of existing wild-endophyte-infected ryegrass (Tables 1 and 2). Although direct drilling into existing pasture after hard

grazing (undersowing) diluted the concentration of lolitrem B compared to untreated nearby pasture (Table 1), this method is not recommended because lolitrem B levels vary between seasons and could increase to levels high enough to cause animal health problems.

#### How to maintain a novel-endophyte pasture

Hay made from wild-endophyte-infected ryegrass with mature seedhead present should not be fed out on AR1-infected ryegrass pastures. Livestock that have been grazing mature ryegrass seedheads containing seed infected with wild endophyte should not be moved directly onto AR1-infected ryegrass pastures. A withholding period of at least 2 days will prevent seed being transferred in cow dung (Burggraaf and Thom, 2002). In addition, maintaining a healthy, vigorous perennial ryegrass pasture will help to reduce the establishment of any wild-endophyte-infected ryegrass plants in the novel-endophyte pasture.

#### Summary of management requirements

1. Select a paddock for renewal.
2. Plan for the establishment of a novel-endophyte pasture.
3. Complete a soil test and apply necessary fertiliser.
4. Prevent natural reseeding of endophyte-infected perennial ryegrass by growing a summer forage crop. If going from grass to grass, ensure that hay is not made off this paddock in the summer prior to renewal.
5. Kill all existing endophyte-infected perennial ryegrass with the correct application rate of herbicide.
6. Deplete levels of buried seed either by ploughing to bury the seed or by a fallow period followed by a second application of herbicide once wild-endophyte-infected ryegrass seedlings have emerged after autumn rain.
7. Sow certified seed to ensure the purity of the novel endophyte.
8. Manage to maintain the established novel-endophyte pasture by preventing the reintroduction of wild-endophyte-infected perennial ryegrass seed in hay, on machinery, or in the dung of livestock

that previously grazed wild-endophyte-infected ryegrass pasture containing mature seedheads.

#### Conclusions

This paper has outlined possible ways to establish novel-endophyte pastures to minimise contamination from wild-endophyte-infected ryegrass. Good paddock preparation will ensure farmers receive the benefits of using novel-endophyte pastures. Ongoing management is necessary to prevent the reintroduction of wild-endophyte-infected ryegrass seed in hay and dung.

#### References

- Bluett, S. J., Burggraaf, V. T., Hume, D. E., Tapper, B. A., and Thom, E. R. 2001. Establishment of ryegrass pastures containing a novel endophyte. *Proc. New Zealand Grassland Association* 63:259–265.
- Burggraaf, V. T., and Thom, E. R. 2000. Contamination and persistence of endophyte-free ryegrass pastures established by spray-drilling and intensively grazed by dairy cows in the Waikato region of New Zealand. *New Zealand Journal of Agricultural Research* 43:163–173.
- Burggraaf, V. T., Thom, E. R. (2002). Minimising endophyte contamination from ryegrass seed in cow dung. *Proc. New Zealand Grassland Association* 64:97–101.
- Fletcher, L. R. 1999. 'Non-toxic' endophytes in ryegrass and their effect on livestock health and production. In *Ryegrass endophyte: an essential New Zealand symbiosis*. Grassland Research and Practice Series 7, New Zealand Grassland Association, pp. 133–139.
- Hume, D. E. 1999. Establishing and maintaining a toxin-free pasture: a review. In *Ryegrass endophyte: an essential New Zealand symbiosis*. Grassland Research and Practice Series 7, New Zealand Grassland Association, pp. 123–132.
- Reed, K. F. M., Leonforte, A., Cunningham, P. J., Walsh, J. R., Allen, D. I., Johnstone, G. R., and Kearney, G. 2000. Incidence of ryegrass endophyte (*Neotyphodium lolii*) and diversity of associated alkaloid concentrations among naturalised populations of perennial ryegrass (*Lolium perenne* L.). *Australian Journal of Agricultural Research* 51:569–578.