# Survival of endophyte-infected ryegrass seed buried in soil

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## Abstract

Emergence of volunteer perennial ryegrass (*Lolium perenne* L.) from seed buried in soil may contribute to the ingress of ryegrass in newly sown pastures. To investigate this, ryegrass seed infected with fungal endophyte (*Neotyphodium lolii*) was buried in nylon bags under pasture at two depths and at two sites (Palmerston North, Lincoln) in early/mid-autumn 1998. Seed bags were removed from the soil at intervals over the course of one year to determine seed viability and presence of endophyte in seedlings. Viability of seed declined rapidly to be 10% 3 months after burial. Further decline in viability was less, so that 12 months after burial 4% of seeds were still viable. Endophyte viability also declined, from 58% infection of seedlings at the time of burial to 21% at 12 months. This was at a slower rate than the decline in seed viability and from what might have been predicted from seed storage experiments. Viability of seed buried at 10 cm was greater than that buried at 3 cm (e.g., means, 10% and 1% after 6 months, respectively). This has implications for cultivation practices before pasture establishment. Seed buried at Lincoln maintained higher viability than seed buried at Palmerston North (e.g., means, 6% and 4% after 6 months, respectively), which was associated with drier soil conditions at Lincoln. Survival of buried seed may therefore be of greater importance in summer-dry east coast regions, compared with moist west coast environments or in wet years. The significance of buried ryegrass seed will depend on the numbers involved, but after 12 months there were still viable seeds left in the soil and some of these were infected with endophyte. This is important for pastures sown with ryegrass that is free of endophyte or infected with a selected endophyte, and for slower establishing grass species such as tall fescue.

**Keywords:** endophyte, endophyte survival, *Lolium perenne, Neotyphodium lolii*, perennial ryegrass, seed burial, seed survival

## Introduction

Ingress of volunteer perennial ryegrass (*Lolium perenne* L.) in newly sown pastures is an important issue for establishment of endophyte-free ryegrass and ryegrass infected with selected endophytes (*Neotyphodium lolii*) (Thom *et al.* 1997). It is also a continuing issue for slower establishing grass species such as tall fescue (*Festuca arundinacea* Schreb.) (Lancashire & Brock 1983; Hume & Lyons 1993), and for ryegrass seed production (Archie & Rowarth 1994). A possible source of ryegrass contamination is through the germination of ryegrass seed that has been buried in the soil, this seed arising from natural reseeding, hay feeding, transfer in dung, etc.

The existence of a reservoir of buried viable seeds is known to occur for many species (Harris 1959; Thompson & Grime 1979). For example, large numbers of buried seeds of fathen (*Chenopodium album* agg.) and wild oats (*Avena fatua* L.) can contribute to these plants being weeds under arable cropping, while buried seeds of annual poa (*Poa annua* L.) and gorse (*Ulex europaeus* L.) contribute to the weed potential of these plants in pastures. Buried seeds may also be important in pasture regeneration, for example, white clover (*Trifolium repens* L.) (Blackmore 1964), and is essential for the survival of an annual species such as subterranean clover (*T. subterraneum* L.) (Chapman 1992). Perennial ryegrass is considered to have “poor” survival as a buried seed in broad ecological terms (Chancellor 1978; Thompson & Grime 1979; Thompson & Grime 1983; Williams 1983), showing little dormancy and few stimulatory requirements for germination, and is therefore a short-lived seed in the soil. Pasture studies in New Zealand hill country (Hume & Barker 1991) and lowland (L’Huillier & Aislabie 1988; van Vught & Thom 1997) have shown that the majority of seed that falls on the soil surface over summer, germinates over the following autumn and winter. Burial of seed may result in greater longevity (Froud-Williams *et al.* 1984) so that viable ryegrass seed may persist beyond the first autumn/winter (Harris 1961), but there is little published data to support or quantify this in New Zealand. Viability of wild-type endophyte in buried seed (van Vught & Thom 1997) is also an issue, particularly when perennial ryegrass is to be resown.

This study assessed the viability of buried ryegrass seed, and endophyte in this seed, with the aim of providing information for farmers on the time required to fallow land before establishing a new pasture.

## Materials and methods

Samples of perennial ryegrass seed were buried under pasture at two depths (3 and 10 cm) at two sites in autumn 1998, with three replications in a randomised block design. One site was at AgResearch, Palmerston North, where seed was buried in a Karapoti silt loam on 16 March 1998, while the other site was at AgResearch, Lincoln, with seed burial on 22 April 1998 in a Templeton silt loam. Pastures were grazed by sheep as part of the normal rotational grazing at each site. Seed was contained in 10 cm × 7 cm nylon bags (50 micron pore size) which allowed free movement of water and micro-organisms, but excluded entry of large invertebrates such as earthworms. Each bag contained 5 g of dry soil from the site of burial, and 0.35 g seed of 'Grasslands Nui' perennial ryegrass that had been harvested in the 1997/98 summer. This seed was infected with its natural wild-type endophyte at the rate of 62% seed-borne endophyte. Seed and endophyte viability was assessed in May 1998 by sowing 0.35 g seed in the glasshouse and counting emerged seedlings to determine % seedling emergence and testing seedlings for endophyte (see below). This showed that on average, each bag contained 146 viable seeds, 58% of which were infected with viable endophyte.

Burial was achieved by removing a 2.5 cm diameter soil core to 10 cm depth, placing a bag in the hole, back filling the hole with the soil until 3 cm from the surface, and then placing another bag in the hole and continuing to fill the hole. As the hole was filled with soil, it was compacted to approximately the same bulk density as the surrounding undisturbed soil. Bags were removed from the soil on eight occasions: at intervals of 1.5 months for the first six months after burial, after which removal occurred at three-month intervals until 12 months after burial. Seed from the bags was then sown in the glasshouse in trays of potting mix and covered by 0.5 cm of sand, and recorded for numbers of seedlings emerged. This represented the number of seeds that would form new plants if seed was placed in conditions suitable for germination and emergence. An assessment of total seed viability, as could have been determined by a tetrazolium test (Lakon 1949), was not carried out. Four to 8 weeks after sowing, seedlings were tested for the presence of endophyte using a polyclonal antibody immunoblot procedure (Gwinn *et al.* 1991).

Seedling emergence and % endophyte presence were analysed with a Generalized Linear Model with binomial errors with the Genstat statistical package (Genstat 5 Committee 1993). Where the trends did not follow a straight line, a non-parametric cubic smoothing spline was used to allow for curvature in the data. Climate data were collected from the Broadfields weather station, 800 m from the seed burial site at Lincoln, and from the AgResearch weather station, 10 m from the seed burial site at Palmerston North.

## Results and discussion

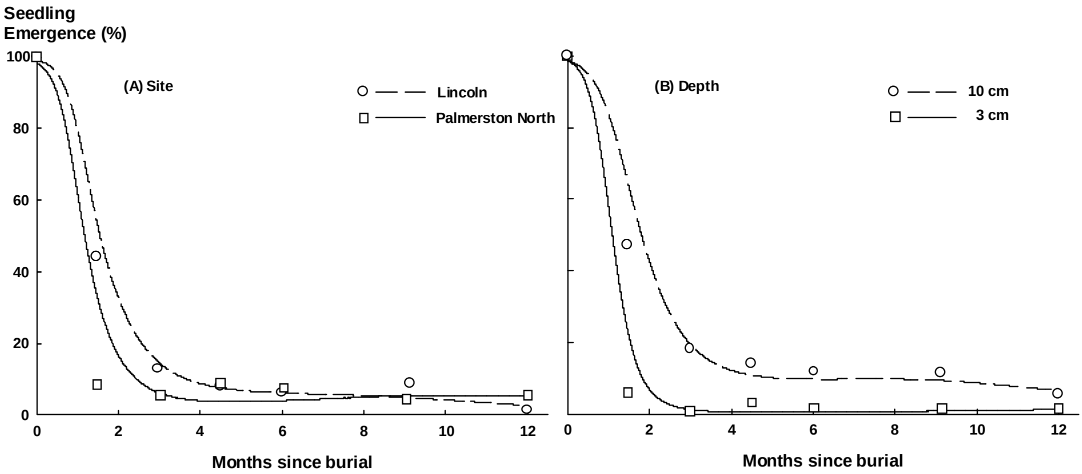
***Seed viability***

Survival of viable seed, as assessed by emergence in the glasshouse, declined rapidly over the first 3 months of burial, followed by a slowing decline over the next 9 months ($P<0.001$) (Figures 1A, B). This required a cubic smoothing spline with 3 degrees of freedom. Mean survival was only 42% after 1.5 months burial and 10% after 3 months. Many other studies have reported similar rapid declines (e.g., Williams 1983). For this reason perennial ryegrass seed has been classified as having short-term dormancy (post-harvest dormancy) forming a transient seed bank present during the summer, with seeds germinating in response to cooler moist conditions in the autumn (Thompson & Grime 1979). As such, ryegrass is adapted to exploit gaps that are created by seasonally-predictable pasture damage and death caused by summer–autumn drought, or by insect pests e.g., porina. Although many of these ryegrass studies have been in northern hemisphere environments, Popay *et al.* (1995) found that seasonal emergence patterns for a range of species in New Zealand were similar to, although more spread out than, those of northern Europe.

Despite the poor initial (3 months) survival of seed in the current study, further declines in survival were low so that there was a small but persistent number of seeds surviving in the soil after 12 months. Some studies have reported similar low survival over several years, e.g., 5% (Harris 1961) (Manawatu, New Zealand) and 0.1% (Roberts 1986) (UK) after 2 years, <1% (Rampton & Ching 1970) (Oregon, USA) and 4% (Lewis 1973) (UK) after 4 years. It is this residual level of surviving seed that when returned to near or at the soil surface, or is already close to the soil surface, may germinate and establish new ryegrass plants. Seed may be transferred up the soil profile through earthworm activity, cultivation, livestock treading or erosion.

Seed that did not germinate in the glasshouse may have decayed through microbial activity while buried in the soil, or imbibed and germinated before removal from the soil as was observed to occur in bags at the 3 cm depth.

**Figure 1** Survival of buried endophyte-infected ryegrass seed (% of viable seed buried) as assessed by seedling emergence in the glasshouse after increasing time of burial at (A) 2 sites, and (B) 2 burial depths.

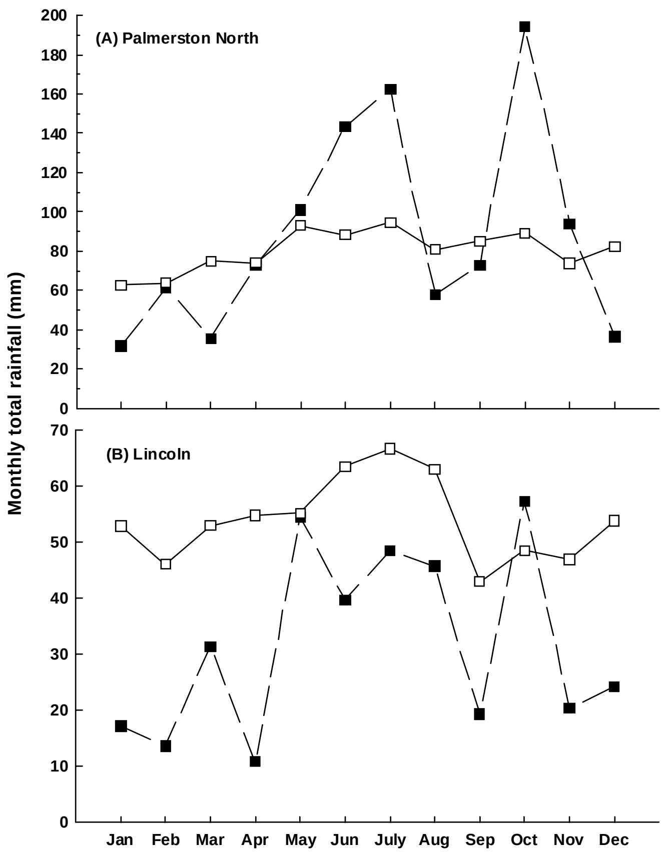



### *Site differences*

Seed survival differed significantly ($P<0.001$) between the sites (Figure 1A), being higher at Lincoln than Palmerston North (e.g., means, 6% and 4% after 6 months, respectively). Several factors can affect survival of buried seed, such as soil type, rainfall/ soil moisture, cultivation, temperature fluctuations, light, and soil oxygen concentrations (Simpson 1990). It is difficult to postulate which factor(s) were responsible for site differences in this study. However, contrasting rainfall pattern was a key difference between these sites in the first autumn/ winter of this study.

Palmerston North (burial on 16 March) was drier and warmer than average during January–March, with rainfall of 129 mm being only 64% of the long-term average (Figure 2A), gravimetric soil moisture approximately 19% (Carran & Theobald unpublished data), and mean temperature 1.3°C above average (17.4°C) for this period. Monthly rainfall then increased to be average or above for the following months, with a resulting increase in soil moisture e.g., 34% on 5 May (Carran & Theobald unpublished data). This was in contrast to Lincoln (burial on 22 April) where

**Figure 2** Monthly rainfall for 1998 (– – ■ – –) and 30-year long-term average (–□–) for (A) Palmerston North and (B) Lincoln.



rainfall was very low from January to April (73 mm, 35% of the long-term average) (Figure 2B), and mean temperatures 2.5°C above average (14.5°C) for this period. Rainfall continued to be low for the rest of 1998 (70% of average), with only May and October having average rainfall. Initial results from further burial of seed in autumn of the following year (unpublished data), show site differences to be reversed, corresponding with a reversal in rainfall pattern and resulting soil moisture compared with the 1998 seed burial. Studies in summer-dry areas of New Zealand for Chilean needle grass (*Stipa neesiana* Trin. & Rupr.) in Marlborough (Bourdôt & Hurrell 1992) and ryegrass in Wairarapa (Hume & Barker 1991) have also found that autumn rainfall had a major influence on the time of seed germination and resulting decline in survival of buried seed. Lower survival of seed with higher rainfall, may be a result of sufficient moisture in the soil for seeds to imbibe and germinate, and/or seeds to rot through greater microbial decay as soil moisture becomes non-limiting.

If rainfall, or more correctly soil moisture, is a major factor determining survival, then summer-dry areas of eastern New Zealand, as typified by Lincoln (mean annual rainfall 647 mm), will typically have higher survival of buried seed than summer-moist areas as typified by Palmerston North (mean annual rainfall 962 mm). Use of irrigation will also affect this. Irrespective of the differing rainfall patterns and the corresponding site differences, it is important to note that seed survival at both sites declined dramatically over the first few months of burial, and from an ecological perspective the differences may not be significant.

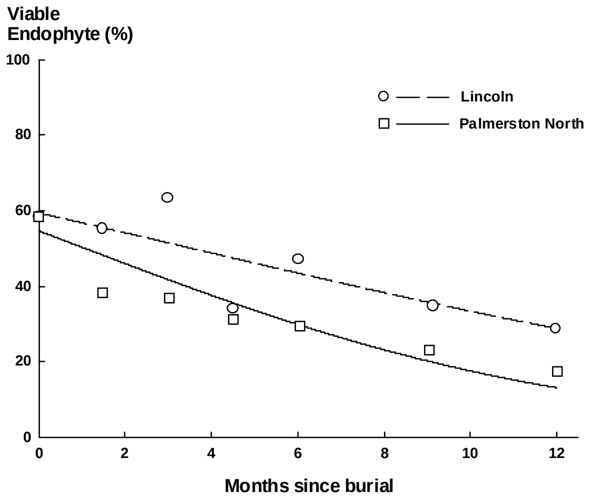
### *Depth of burial*

Depth of burial affected seed survival (P<0.001), with seed buried at 3 cm having a lower survival than at 10 cm burial (e.g., means, 1% and 10% after 6 months, respectively) (Figure 1B). The effect of depth was much larger than the effect of site. There was no significant interaction between depth and site effects (P=0.118), implying that the depth effects were consistent at both sites. The effect of depth is a general phenomenon for many seeds (e.g., Froud-Williams *et al.* 1984), and may be due to lower temperatures, lower daily temperature fluctuations, and less light and oxygen at greater depths of burial (Simpson 1990). Other studies with perennial ryegrass have not shown the same response to burial (e.g., Lewis 1973).

### *Endophyte*

The proportion of seedlings infected with endophyte (Figure 3) declined linearly (P<0.001) with increasing time of burial (58% infected at the time of burial compared with 20% 12 months after burial). This decline was less at Lincoln than Palmerston North (29% and 13% at 12 months, respectively), similar to the differences between sites for seed survival. The Y-intercepts for the sites were significantly different (P=0.014), while slopes were similar (P=0.161). This was primarily due to a greater decline in endophyte viability between burial and 1.5 months for Palmerston North than Lincoln, after which declines (slopes) in viability were similar. Burial depth had no significant effect (P=0.474). Van Vught & Thom (1997) also recorded viable endophyte (60%) in buried ryegrass seed in the Waikato in autumn, but initial % endophyte infection and length of burial were unknown. The survival of endophyte in the current study, and from further burial of seed in autumn of the following year (unpublished data), was better than what might have been predicted from seed storage experiments. In such storage experiments, endophyte typically loses viability well before seeds lose viability (Rolston *et al.* 1986; Welty *et al.* 1987; Hare *et al.* 1990). Conditions in the soil may differentially favour survival of seed with viable endophyte, for example, through imparting a protective advantage to the seed as has been indicated by studies of *Pythium* infection (Gwinn & Batzer unpublished data) and seedling emergence (Quigley *et al.* 1993). However, any such advantages must be marginal as most of the seed loses viability, whether infected with viable endophyte or not.

**Figure 3** Endophyte presence in seedlings (%) after increasing time of burial at 2 sites.



## Implications

Until the results of further seed burial experiments at other locations and in other years are available, the results are limited to the sites used and the environmental conditions prevailing during this study. Results also may vary between cultivars (Wiesner & Grabe 1972) and the environmental conditions during seed development and maturity (Wiesner & Grabe 1972) as this can affect dormancy of ryegrass seed. The technique of burying seed in bags, also excludes invertebrates such as earthworms that may ingest seed, thus reducing its viability (McRill & Sagar 1973) and transferring seed around the soil profile. Endophyte presence may also deter some insects from consuming seed with a preference for endophyte-free seed (Popay unpublished data), thus favouring the survival of endophyte-infected seed.

This study has highlighted that ryegrass seed, and endophyte, can survive for up to 12 months of burial in the soil. Thus if fallowing, cropping or grazing are used to prevent natural reseeding for 12 months, it cannot be assumed that there will be no viable ryegrass seed in the soil. The impact this may have on resulting contamination of pastures will depend on the quantity of seed that enters the soil, and in part, on what cultivation techniques are used (Archie & Rowarth 1994). Cultivation may return seed to or near to the soil surface, and this seed may then successfully establish new ryegrass plants.

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