Epichloë endophyte survival in rye (*Secale cereale*) and perennial ryegrass (*Lolium perenne*) seeds under different storage conditions

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Abstract

Epichloë fungal endophytes are an important symbiont of some temperate forage grasses, providing the plant host with protection from biotic and abiotic stresses. Recent research has focussed on applying the potential benefits of the asexual form of this endophyte to cultivated cereal crops. Cereals are not known to naturally host *Epichloë* endophytes but can be artificially inoculated with selected fungal strains isolated from *Elymus* and *Hordeum* species. A critical aspect of a successful association is the ability of these obligate mutualistic endophytes to transmit through the seed and colonise the resulting offspring. Within agricultural and cropping systems, seed can be stored for varying lengths of time before being sown. The ability of these fungal endophytes to survive in stored seed and colonise the next generation of plants is an important consideration.

Optimal storage conditions required for *Epichloë* survival in seed of perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) are well documented, but little is known about endophyte survival within stored cereal grain, nor the conditions that are required for maximising its survival. Rye (*Secale cereale* L.) grain colonised with endophyte strain AR3002 and perennial ryegrass seed colonised with AR1 endophyte were stored under different temperature and humidity conditions, with the survival of both host and endophyte evaluated over a 2-year period.

Under controlled conditions of 0°C and a relative humidity of 24%, the endophyte and host maintained stable levels of viability for both species for the duration of the trial. In environments where both the temperature and relative humidity were not controlled and the seed was exposed to ambient conditions, the endophyte generally declined rapidly within 12-18 months, although in most cases the seed itself remained viable, for both rye and perennial ryegrass seed. The endophyte associated with rye responded in a similar way to endophyte associated with perennial ryegrass in these storage environments. This understanding provides guidance on seed storage conditions required to ensure sustained endophyte viability and also identifies timeframes in which endophytic grain will need to be resown if held at ambient conditions. Further work will be required to determine if similar limitations are applicable to *Epichloë* endophytes in wheat.

Keywords: endophyte viability, *Epichloë bromicola, E. festucae* var. *lolii*, germination, seed

Introduction

Epichloë fungal endophytes are an important symbiont of some temperate forage grasses. In New Zealand pastoral agricultural systems, selected asexual *Epichloë* strains in perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) are essential for improving the productivity and persistence of pastures, through providing the host with protection from biotic and abiotic stresses while having little to no negative impacts on livestock (Johnson et al. 2013). A critical aspect of a successful association is the ability of the endophyte to transmit through the seed and colonise the resulting offspring. The effectiveness of this technology on-farm depends on the endophyte remaining viable in seed, as commercial endophyte strains are sold in elite cultivars (Johnson & Caradus 2019). There is a requirement under some New Zealand licence agreements for endophyte viability

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to be greater than 70% in commercial seed sold as containing a selected *Epichloë* endophyte at the time of sale to the retailer (Caradus 2023). Within agricultural and cropping systems, seed can be stored for various lengths of time before being sown, making it essential that the endophyte can survive in stored seed to persist and colonise the next generation of plants.

Studies have shown that endophyte viability in stored seed of ryegrass (*L. perenne*, *Lolium multiflorum* Lam.) and fescues (*F. arundinacea*, *Festuca pratensis* Huds.) will decline before the seed loses viability (Gundel et al. 2009; Hume et al. 2013; Bylin et al. 2016). Factors influencing this decline include the temperature and humidity of the storage environment, length of storage, initial seed moisture content, grass species and cultivar, seed line, endophyte strain and packaging (Rolston et al. 1986; Welty et al. 1987; Gundel et al. 2009; Hume et al. 2011; Hume et al. 2013; Bylin et al. 2016). Recommended conditions for preserving *Epichloë* spp. in stored ryegrass seed include starting with a seed moisture below 8% and storing in ≤5˚C and ≤30% relative humidity (RH) (Hume et al. 2013), however these conditions are often unrealistic to achieve at commercial scale.

More recent research has focussed on applying the potential benefits of *Epichloë* spp. to cereal crops. *Epichloë* spp. are not known to naturally colonise cultivated cereal grasses but are present in grass species within the genera *Elymus* and *Hordeum* (Card et al. 2014) and selected fungal strains have been able to form stable associations with rye (*Secale cereale* L.) when inoculated artificially (Simpson et al. 2014). Endophyte presence in modern cereals has been shown to enhance the host plants' natural defences to biotic and abiotic stresses, primarily through the production of secondary metabolites (Hume et al. 2018; Wang et al. 2022; Popay et al. 2023). Given recent advances in creating these artificial *Epichloë* associations with modern cereals, and the prospect of commercial application of this technology, the stability of these associations under differing storage conditions requires investigation.

In this study, the potential for *Epichloë* endophytes to survive in rye and perennial ryegrass under different ambient and controlled temperature and RH storage conditions was examined. Optimal storage conditions for *Epichloë* endophytes in cereal grain is discussed.

Materials and Methods

Germplasm

Two host grass species containing associated endophyte strains, and endophyte-free comparators (four combinations in total), were used for this trial (Table 1). The rye host was cv. 'Rahu' associated with *E. bromicola* strain AR3002, originally isolated from *Elymus dahuricus* Turcz. ex Griseb. (Simpson et al. 2024). The perennial ryegrass host was cv. 'GA66' associated with *E. festucae* var. *lolii* strain AR1, originally isolated from perennial ryegrass (Johnson et al. 2013). In both cases, the endophytes had previously been artificially inoculated into the host germplasm and undergone several cycles of seed production. All seed lines used in this experiment had been stored since harvest at 0˚C and 30% RH in the Margot Forde Genebank at Palmerston North, the national genebank of grassland and forage plants in New Zealand.

Storage sites

Three AgResearch locations in New Zealand were used for this trial. The northernmost site was Hamilton (-37.776647, 175.309628, 45 m elevation), the central site was Palmerston North (-40.380430, 175.612428, 34 m elevation) and the southernmost site was Lincoln (-43.628087, 172.469728, 18 m elevation). At each site, seed was stored within outbuildings or sheds without controlled temperature or RH, similar to buildings used for commercial seed storage. Two additional storage environments at Palmerston North were included in this trial – the Margot Forde Genebank, and a metal container with a tightly fitting lid (opened and resealed for each 3-monthly sampling) stored at ambient conditions alongside the seed in the outbuilding. Seed was despatched to each site in paper bags and placed in the described storage conditions on 16 November 2021.

Storage conditions

At each site a Digitech QP-6013 data logger recorded temperature and RH at 10-minute intervals. The conditions at all sites were different from each other ($P<0.05$). At the ambient storage sites, temperature and RH fluctuated with seasons (Figures 1 and 2), summers being warm with low RH while winters were cool with higher RH. The northernmost site at Hamilton recorded the highest mean temperature (17.6°C) and mean RH (69.6%), with the central site at Palmerston North having slightly lower mean temperature (17.1°C) and lower mean RH (65.8%), and the southernmost site at Lincoln being the coolest (14.8°C) with the lowest RH (65.5%).

The controlled temperature/RH site in the Margot Forde Genebank recorded a mean temperature of -0.4°C and mean RH of 23.5% without seasonal fluctuations (Figures 1 and 2). Seeds stored in the container at Palmerston North experienced a similar mean temperature (17.0°C) to the seed stored outside of the container (17.1°C), with similar fluctuations (Figure 1). However, the mean RH over the trial period within

Table 1 Host and endophyte information for seed used in storage trial.

Host species	Endophyte status	Harvest year	Thousand seed weight (g)	Seed weight despatched to each storage site (g)	Seed weight tested each 3-months (g)
Secale cereale	AR3002	2018	16.1	104	13.0
Secale cereale	Endophyte-free	2018	23.5	104	13.0
Lolium perenne	AR1	2021	2.3	84	10.5
Lolium perenne	Endophyte-free	2018	2.6	84	10.5

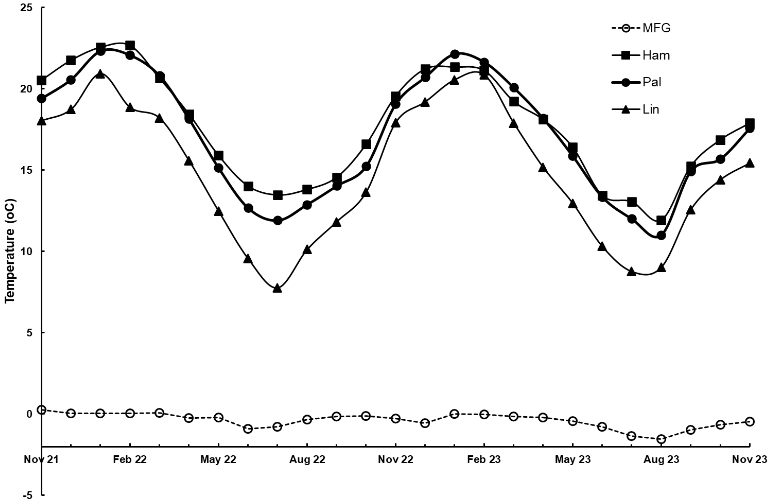

Figure 1 Monthly mean temperature at each storage site. MFG = Margot Forde Genebank, Ham = Hamilton, Pal = Palmerston North, Lin = Lincoln. The container storage environment has not been included in this figure because it was obscured by the Palmerston North data line.

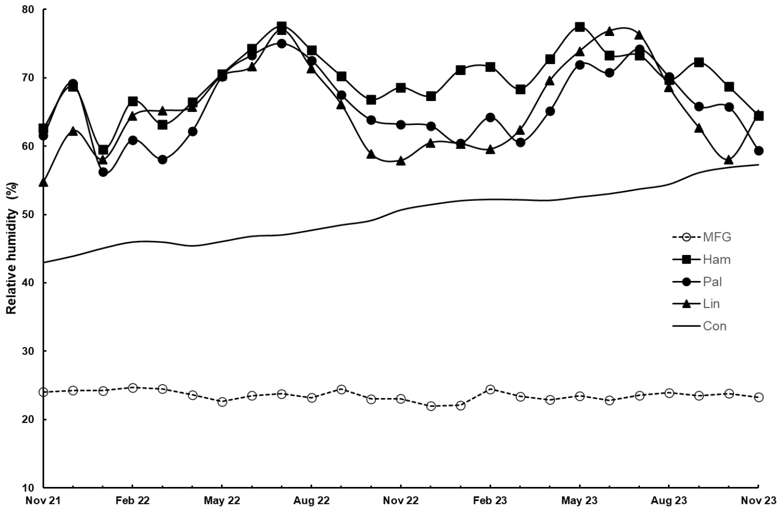

Figure 2 Monthly mean relative humidity at each storage site. MFG = Margot Forde Genebank, Ham = Hamilton, Pal = Palmerston North, Lin = Lincoln, Con = metal container at Palmerston North.

the container (50.0%) was much lower than the ambient environment (65.8%), and with less fluctuations, but there was a steady rise of 14.3 percentage units from 43.0% to 57.3% over the trial period (Figure 2).

Methodology

At 3-monthly intervals from 16 November 2021 over a 2-year period, the seed in each bag was mixed thoroughly and a sample removed for testing (Table 1). Seed samples were sealed within two plastic bags at the storage site, then sent (by overnight courier if sent from outside Palmerston North) to Palmerston North for testing. A 10 g sub-sample from each seed sample was tested for moisture content. Ryegrass seed was weighed before and after drying at 130˚C for 2 hours. Rye grain was finely ground and then weighed before and after drying at 130°C for 2 hours. Seed moisture level was calculated and expressed as a fresh weight percentage using the formula [(g fresh weight) - (g dried weight)] / (g fresh weight) (ISTA 2021).

From the remaining seed, up to 100 seeds were sown into seedling trays (cell dimensions: 32 mm x 32 mm x 40 mm) filled with potting mix and germinated in a glasshouse. Trays were watered as required and after 6-8 weeks the emergence percentage was determined by counting the plant numbers in each tray. At the same time, endophyte survival was determined using an immunoblot assay (Simpson et al. 2012) on a single tiller from each plant.

Statistics

Seed emergence, endophyte viability and seed moisture content data were analysed using linear regression modelling, with the factors of grass host, site, endophyte status and time (months). Time was modelled as a 4th order polynomial. For the germination model, two-way and three-way interaction terms were included in the model. Two-way interaction terms were grass host and site, site and endophyte status, grass host and time, site and time, and time and endophyte status. Three-way interaction terms were site, grass host and endophyte status; site, time and endophyte status; site, grass host and time; and grass host, time and endophyte status. For the endophyte model, the two-way interaction terms of site and grass host, grass host and time, site and time, and the three-way interactions of site, grass host and time were included in the final model. For the model comparing endophyte and plant survival, the % emergence and % viable endophyte data for each sample was stacked and a new factor called organism was added to the above model used for seed emergence, in addition to its interaction with other covariates and factors. The temperature and RH data were analysed with generalised additive models using the mgcv r package version 1.9.1 and the factors were site and time (days). All analyses were conducted in R version 4.4.2.

Results

Emergence

For the regression model, the Margot Forde Genebank provided the baseline site. At ambient sites, emergence levels varied between grass hosts ($P<0.001$), absence/presence of endophytes ($P=0.004$) and sites ($P<0.01$) (Figure 3). There was an interaction between the site and time, with emergence declining over time ($P<0.05$).

On average, ryegrass had a higher emergence rate than rye ($P<0.001$), this difference being greater in seed stored at more northern sites in New Zealand. The sharpest declines in emergence occurred at Hamilton for rye, with levels for both the endophyte-infected and endophyte-free lines dropping from a starting level of ≥85% to below 5% within 21 months of storage. Seed at Palmerston North showed declines in emergence for both endophyte-infected and endophyte-free rye lines to below 50% within 24 months of storage, while at Lincoln only the endophyte-infected rye line declined from a starting level of 85% to below 70% within 24 months of storage. Emergence levels for ryegrass remained above 90% at all storage sites for the duration of the trial, except for Hamilton, where emergence for the endophyte-infected and endophyte-free lines had dropped to 71% and 87% respectively within 24 months of storage (Figure 3).

The endophyte-infected lines had greater declines in emergence rate than endophyte-free lines at Lincoln for rye ($P<0.02$) and Hamilton for ryegrass ($P<0.02$) (Figure 3). In the container at Palmerston North, there were no changes in emergence rate over time ($P>0.05$) or differences between host grasses ($P>0.05$).

Endophyte

For the regression model, the Margot Forde Genebank provided the baseline site. At ambient sites, viable endophyte levels varied between sites ($P<0.001$) and there was an interaction between site and time ($P<0.05$), with endophyte viability declining over time (Figure 4). Grass species was not associated with endophyte viability ($P=0.278$). Endophyte declined at similar rates at the Hamilton, Palmerston North and Lincoln sites (all $P<0.001$), with least decline in the container at Palmerston North ($P<0.001$) (Figure 4).

At ambient sites all lines declined in viable endophyte to ≤1% by the conclusion of the trial (Figures 3 and 4) and the level of viable endophyte in the germplasm declined before the host viability declined (Figure 3). Endophyte levels remained above 70% for a

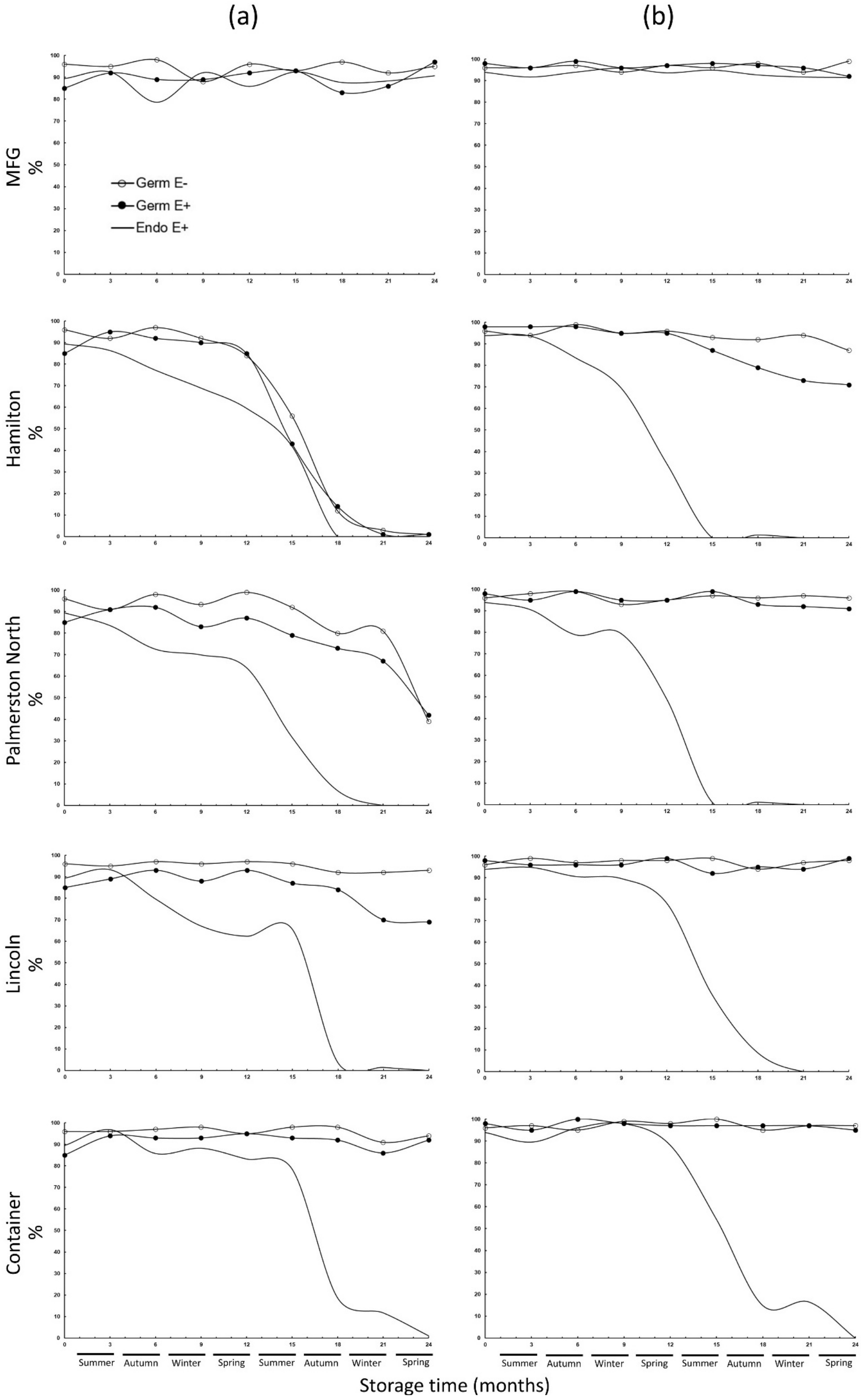

Figure 3 Host and endophyte viability (%) for (a) rye and (b) ryegrass germplasm stored at five sites for 24 months from November 2021. Germ E- = endophyte-free host emergence, Germ E+ = endophyte-infected host emergence, Endo E+ = endophyte viability. MFG = Margot Forde Genebank. Standard error for E+ vs E- is 1.64. Standard error for plant vs endophyte survival is 0.96.

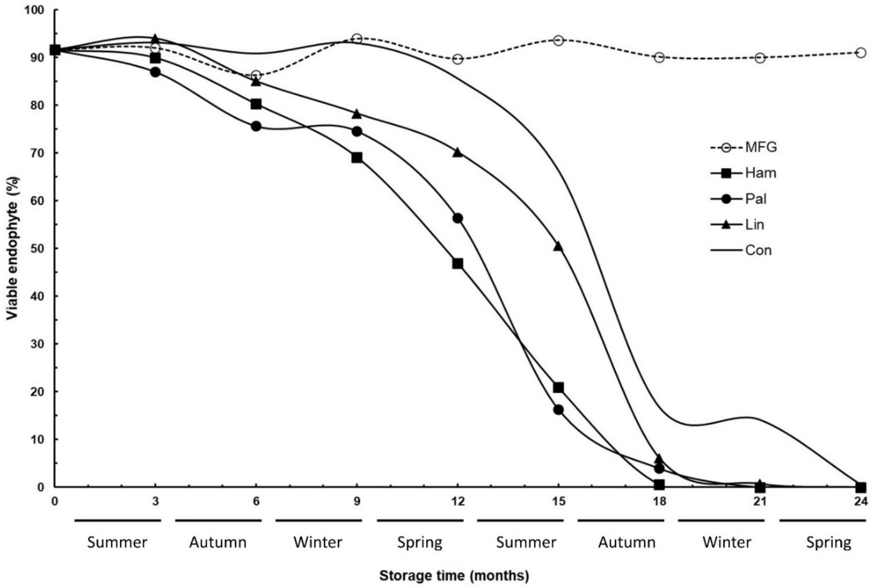

Figure 4 Endophyte viability (%) for rye and ryegrass germplasm (combined mean) stored at five sites for 24 months from November 2021. MFG = Margot Forde Genebank, Ham = Hamilton, Pal = Palmerston North, Lin = Lincoln, Con = metal container at Palmerston North. Standard error is 4.46.

minimum of 6 months of storage, regardless of storage environment (Figure 4).

Seed moisture

For the regression model, the Margot Forde Genebank provided the baseline site. Moisture levels varied between site ($P<0.05$) and time ($P<0.01$), but not endophyte status ($P=0.650$). Perennial ryegrass seeds had a lower overall moisture content than rye grain (mean difference 1.5%, $P<0.001$). There was an interaction between site and time for the Palmerston North, Hamilton and Lincoln sites (all $P<0.05$) compared with the Margot Forde Genebank (Figure 5). Seed in the Margot Forde Genebank had a stable moisture content while seed in the container had a gradual increase of 1.2 percentage points over the trial period ($P<0.01$). At the other ambient sites, there were large cyclic fluctuations in seed moisture over the trial period (Figure 5).

Discussion

During seed storage, *Epichloë* endophyte associated with a rye host exhibited similar characteristics to those of endophyte associated with ryegrass, that is, endophyte viability declined in seed before the seed died, and declines were greatest in uncontrolled environments with higher temperatures and RH. This general response of stored endophyte-infected seed has been reported in several other studies with ryegrasses, tall fescue and meadow fescue (Welty et al. 1987; Gundel et al. 2009; Hume et al. 2011; Hume et al. 2013; Bylin et al. 2016).

At Hamilton and Palmerston North under ambient storage conditions, emergence declined at a greater rate for rye than ryegrass, while at all sites the viability of endophyte associated with rye decreased in a similar way to that of endophyte associated with ryegrass. Endophyte decline in stored ryegrass seed has previously been described as a logistic relationship (Hume et al. 2013), that is, “a plateau or slow decline at first, then a rapidly increasing decline, followed by a plateau as the proportion of seeds with viable endophyte approaches zero”. In the present study, a similar effect was observed for endophyte in both host species (Figure 4). This indicates that the *Epichloë* endophyte was able to form a similarly strong association with rye grain when compared to ryegrass seed, despite being an artificially inoculated endophyte derived from a grass species from a different genus to that of rye.

The temperature and RH of a storage environment has a major impact on endophyte viability, and to a lesser extent seed emergence. Under the controlled conditions of low temperature (0°C) and low RH (24%) in the Margot Forde Genebank, host and endophyte viability were maintained for both rye and ryegrass throughout the duration of this experiment. This confirms that

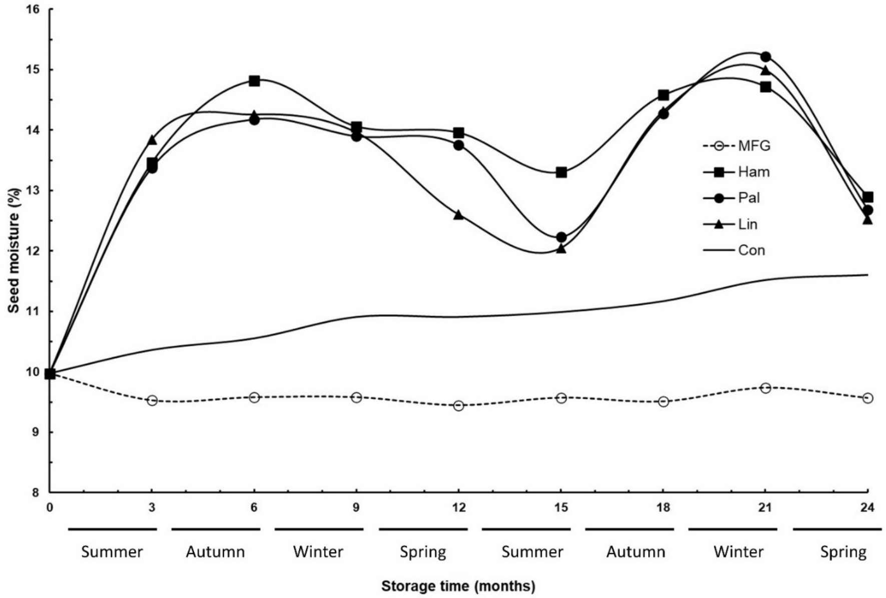

Figure 5 Mean seed moisture content (%) of rye and ryegrass germplasm (combined mean) stored at five sites for 24 months from November 2021. MFG = Margot Forde Genebank, Ham = Hamilton, Pal = Palmerston North, Lin = Lincoln, Con = metal container at Palmerston North. Standard error is 0.259.

Epichloë endophyte associated with rye grain can be maintained for multiple years under these favourable conditions.

Previous studies have suggested that temperature is the main factor influencing endophyte survival during storage under certain conditions (Welty et al. 1987; Hume et al. 2011; Hume et al. 2013; Bylin et al. 2016). In the container storage treatment, the endophytic seed and grain were in a fluctuating temperature environment similar to the Palmerston North site but experienced a comparatively low RH, which was gradually increasing over time with only minor fluctuations. This lower RH in the container was associated with a lower seed moisture content, and potentially greater seed emergence levels, for rye grain stored in the container. Rye grain showed no decline in emergence when stored within the container, compared with a decline to below 50% for grain stored in the ambient Palmerston North treatment. In both environments, endophyte levels declined to ≤1% at the conclusion of the trial, lending further weight to previous assertions on the strong influence of temperature on endophyte survival.

However, endophyte levels of grain and seed in the container, which experienced the lowest RH of all the ambient sites, showed the least decline when compared with the other ambient treatments. Further, endophyte decline at the Lincoln site, which experienced the lowest ambient temperatures with seasonal RH fluctuations, was more rapid than the container treatment. This demonstrates that RH, and its effect on seed moisture content, also played a significant role in the loss of endophyte viability during storage.

The measured RH within the container, and the corresponding seed moisture of the stored seeds, both exhibited a steady rise throughout the trial. This is likely due to the container being opened at 3-monthly intervals to remove a seed sample and the container lid not being completely sealed over the duration of the trial.

The moisture content of seed equilibrates with the RH of the storage environment (Hume et al. 2013), and high levels of seed moisture during storage can be detrimental to endophyte survival (Welty et al. 1987). It has been previously shown that endophyte viability in perennial ryegrass seed declines within 12 months when seed moisture is above 11.3% (Rolston et al. 1986), and endophyte longevity is increased during storage when seed moistures are below 10-12% at temperatures below 20ºC (Hume et al. 2013). In the current study, at ambient sites, seed moisture content followed a similar seasonal fluctuation to that for RH. From the time that seed was despatched there was an increase in seed moisture level at all ambient sites (excluding the container treatment) to above 13% within the first 3 months of storage, and then remained above that level for the following 6-9 months of storage. After 9-12 months of storage, viable endophyte levels started rapidly declining at the ambient sites where temperature and RH measurements were the highest.

Gundel (2009) reported that for annual ryegrass (*Lolium multiflorum* L.) *Epichloë* presence could affect seed viability under certain conditions. In the current study there were differences identified in seed viability levels between endophyte-infected and endophyte-free lines at Lincoln for rye and Hamilton for ryegrass (Figure 3). While these differences could be attributed to *Epichloë* endophyte presence, it could equally be a function of different seed lots, for example, the difference in thousand seed weight between endophyte-infected and endophyte-free lines used in this study, or in the case of ryegrass, a different harvest year.

Overall**,** these results show that *Epichloë* residing within perennial ryegrass seed and rye grain will remain viable under low temperature and low RH storage, and will degrade rapidly in elevated temperature and humidity environments. This has implications for treatment of seed when it moves from the wholesaler into the retail chain. Proprietary seed companies selling endophytic grass seed are committed to ensuring that at least 70% of seed will be infected with live endophyte at the time of sale to the retailer (Caradus 2023). Endophyte viability tests are considered valid for 6 months, but after that time the way in which seed is stored will likely impact, and could result in, reduced levels of viable endophyte in seed. Endophyte levels in the study reported here remained above the 70% industry standard for pasture grasses for a minimum of 6 months of storage, regardless of species or storage environment (Figure 4). However, once seeds enter the rapid decline phase, substantial endophyte losses can occur within a 3–6 month time-frame.

Rolston and Agee (2007) reported that, for perennial ryegrass and tall fescue, seed containing *Epichloë* endophyte should be treated as a high-value perishable product within the supply chain. Quality control measures such as regular testing, using appropriate packaging, and adhering to strict production and storage protocols, were recommended to ensure seed lines delivered a high level of viable endophyte to the end-user. The results presented here suggest that, overall, *Epichloë* endophyte in rye exhibits storage characteristics similar to those found in endophyte-infected grass seed, and therefore similar recommendations are likely to apply to rye, and perhaps other cereals such as wheat.

Conclusions

This study has shown that optimal conditions for ensuring *Epichloë* endophyte viability in seed of ryegrass and tall fescue during long-term storage, i.e., reduced temperature and humidity, will have a similar effect for endophyte in rye grain. As results were limited to assessing a single endophyte strain in a single cultivar, more work is warranted to determine whether this is a repeatable effect. Similarly, further investigations will be needed to determine whether comparable responses are evident for *Epichloë* endophytes in wheat.

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REFERENCES

Bylin AG, Card SD, Hume DE, Lloyd-West CM, Huss-Danell K. 2016. Endophyte storage and seed germination of *Epichloë* - infected meadow fescue. *Seed Science and Technology 44*: 138-155. https://doi.org/10.15258/sst.2016.44.1.21

Caradus JR. 2023. *Epichloë* fungal endophytes–a vital component for perennial ryegrass survival in New Zealand. *New Zealand Journal of Agricultural Research 67*: 451-468. https://doi.org/10.1080/00288233.2023.2170426

Card SD, Faville MJ, Simpson WR, Johnson RD, Voisey CR, de Bonth ACM, Hume DE. 2014. Mutualistic fungal endophytes in the *Triticeae* - survey and description. *FEMS Microbiology Ecology 88*: 94-106. https://doi.org/10.1111/1574-6941.12273

Gundel PE, Martinez-Ghersa MA, Garibaldi LA, Ghersa CM. 2009. Viability of *Neotyphodium* endophytic fungus and endophyte-infected and noninfected *Lolium multiflorum* seeds. *Botany 87*: 88-96. https://doi.org/10.1139/B08-119

Hume DE, Schmid J, Rolston MP, Vijayan P, Hickey MJ. 2011. Effect of climatic conditions on endophyte and seed viability in stored ryegrass seed. *Seed Science and Technology 39*: 481-489. https://doi.org/10.15258/sst.2011.39.2.19

Hume DE, Card SD, Rolston MP. 2013. Effects of storage conditions on endophyte and seed viability in pasture grasses. Presented at the *22nd International Grassland Congress*, Sydney, Australia. 15-19 September. Pp. 405-408.

Hume DE, Drummond JB, Rolston MP, Simpson WR, Johnson RD. 2018. *Epichloë* endophyte improves agronomic performance and grain yield of rye (*Secale cereale*). Presented at the *10th International Symposium on Fungal Endophyte of Grasses*, Salamanca, Spain. 18-21 June.

ISTA (2021). *International Rules for Seed Testing*. Richtiarkade 18, CH-8304 Wallisellen, Switzerland.

Johnson LJ, de Bonth ACM, Briggs LR, Caradus JR, Finch SC, Fleetwood DJ, Fletcher LR, Hume DE, Johnson RD, Popay AJ, Tapper BA, Simpson WR, Voisey CR, Card SD. 2013. The exploitation of epichloae endophytes for agricultural benefit. *Fungal Diversity 60*: 171–188. https://doi.org/10.1007/s13225-013-0239-4

Johnson LJ, Caradus JR. 2019. The science required to deliver *Epichloë* endophytes to commerce. In: Hodkinson TR, Doohan FM, Saunders MJ, Murphy BRT. Eds. *Endophytes for a growing world*. Cambridge, UK: Cambridge University Press, pp. 343-370. https://doi.org/10.1017/9781108607667.017

Popay AJ, Jensen JG, Simpson WR, Mace WJ, Somchit C. 2023. Translocation of loline alkaloids in *Epichloë*-infected cereal and pasture grasses: What the insects tell us. *Journal of Fungi 9*: 96. https://doi.org/10.3390/jof9010096

Rolston MP, Hare MD, Moore KK, Christensen MJ. 1986. Viability of *Lolium* endophyte fungus in seed stored at different moisture contents and temperatures. *New Zealand Journal of Experimental Agriculture 14*: 297-300. https://doi.org/10.1080/03015521.1986.10423042

Rolston MP, Agee C. 2007. Delivering quality seed to specification – the USA and NZ novel endophyte experience. Presented at the *6th International Symposium on Fungal Endophytes of Grasses,* Christchurch, New Zealand. 25-28 March 2007. Pp. 229-231. https://doi.org/10.33584/rps.13.2006.3065

Simpson WR, Schmid J, Singh J, Faville MJ, Johnson RD. 2012. A morphological change in the fungal symbiont *Neotyphodium lolii* induces dwarfing in its host plant *Lolium perenne*. *Fungal Biology 116*: 234-240. https://doi.org/10.1016/j.funbio.2011.11.006

Simpson WR, Faville MJ, Moraga RA, Williams WM, McManus MT, Johnson RD. 2014. *Epichloë* fungal endophytes and the formation of synthetic symbioses in Hordeeae (=Triticeae) grasses. *Journal of Systematics and Evolution 52*: 794-806. https://doi.org/10.1111/jse.12107

Simpson WR, Tsujimoto H, Hume DE, Johnson RD. 2024. Alien chromatin from Hordeeae grasses enhances the compatibility of *Epichloë* endophyte symbiosis with the hexaploid wheat *Triticum aestivum*. *Journal of Fungi 10*: 384. https://doi.org/10.3390/jof10060384

Wang Z, Liu J, White JF, Li C. 2022. *Epichloë bromicola* from wild barley improves salt-tolerance of cultivated barley by altering physiological responses to salt stress. *Frontiers in Microbiology 13*: 1044735. https://doi.org/10.3389/fmicb.2022.1044735

Welty RE, Azevedo MD, Cooper TM. 1987. Influence of moisture content, temperature and length of storage on seed germination and survival of endophytic fungi in seeds of tall fescue and perennial ryegrass. *Phytopathology 77*: 893-900. https://doi.org/10.1094/Phyto-77-893