Effects of storage conditions on endophyte and seed viability in pasture grasses

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Abstract. Several important temperate pasture grasses have co-evolved with mutualistic Epichloë fungal endophytes. These endophytes impart beneficial attributes to their host as they enhance the fitness of the grass when under biotic and abiotic stresses. The asexual species of these fungi (formerly classed as Neotyphodium) are obligate symbionts, and efficiently colonise newly formed tillers and infect seed by direct colonisation of the embryo. These endophytes are strictly seed transmitted. Survival of the fungus in this seed is therefore critical for the dissemination of endophyte-infected seed to grassland farmers. Longevity of endophyte in stored seed is primarily determined by the length of storage, temperature, and relative humidity as this is in equilibrium with seed moisture. Elevated temperature and relative humidity both reduce endophyte viability. The relative importance of each of these environmental parameters is unclear. Longevity may be further modified by grass species, cultivar, seed lot, and endophyte strain. Valuable seed requiring long term storage can utilise controlled storage facilities where temperature is preferably ≤5°C and relative humidity ≤30% (seed moisture <8%). For large quantities of commercial seed, moisture barrier packaging can be used.

Keywords: Festuca arundinacea, Lolium perenne, Lolium hybridum, Lolium multiflorum, Epichloë, Neotyphodium.

Introduction

Many temperate forage grasses have co-evolved with endophytic fungal symbionts of Epichloë, including asexual morphs formerly classified in the form genus Neotyphodium (Schardl et al. 1997). Of greatest significance to agriculture have been the asexual species that infect important pastoral grasses such as tall fescue (Festuca arundinacea Schreb.), meadow fescue (F. pratensis Huds.), and ryegrasses [perennial, annual/Italian, perennial x Italian hybrids (L. perenne L., L. multiflorum Lam., L. boucheanum Kunth syn. L. hybridum Hausskn.)]. When these grass species are infected with Neotyphodium endophytes, mutualistic, non-symptomatic associations are formed (Schardl 2001). The fungus (obligate symbionts) obtains a suitable habitat, nutrition from the plant and an efficient means of dissemination to successive host generations as it infects newly formed tillers and seed. The grass host benefits through increased tolerance to biotic and abiotic stresses, notably insect predation and drought stress (Malinowski and Belesky 2000; Popay and Bonos 2005). While such mutualistic associations are critical in many pastoral systems for plant productivity, some strains of endophyte produce secondary metabolites that cause ill health and productivity losses in livestock grazing these pastures. To mitigate the effects on livestock, but still gain livestock grazing these pastures. To mitigate the effects on livestock, but still gain agriculture and nutrition for the developing embryo and as this structure differentiates and expands, the endophyte is left in the nucellus remnants, now a thin membranous layer of crushed cells. At seed maturity, the

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fungal growth within the seed. This is generally achieved by infecting the seed at the time of harvesting. Once harvested, the infected seed can be stored in seed banks for future use. The endophyte can remain viable in stored seed for several years, depending on environmental conditions such as temperature and humidity. The endophyte is capable of colonising the seed coat and the pericarp, which are the outer layers of the seed. This colonisation can lead to a reduction in seed vigour, as the endophyte can compete with the host plant for nutrients and water. The endophyte can also trigger the production of defensive compounds in the host plant, which can be detrimental to the host's growth and development.

It is important to note that the endophyte is not pathogenic to the host plant. The endophyte is expected to remain viable in stored seed for several years, provided that optimal storage conditions are maintained. The endophyte can remain viable in stored seed for several years, depending on environmental conditions such as temperature and humidity. The endophyte is capable of colonising the seed coat and the pericarp, which are the outer layers of the seed. This colonisation can lead to a reduction in seed vigour, as the endophyte can compete with the host plant for nutrients and water. The endophyte can also trigger the production of defensive compounds in the host plant, which can be detrimental to the host's growth and development.

Commercialisation of endophyte-infected seed

Marketing endophyte-infected seed has added an extra layer of complexity and quality assurance to the seed production and supply chain (Rolston and Agee 2007). Viability of endophyte is a key parameter, which under licence agreements for selected endophytes such as AR1, AR37 and AR542 is required to be >70% (Hume and Barker 2005). It has been advocated that seed production companies and the whole supply chain, including farmers, treat endophyte-infected seed as a perishable high-value product and so take appropriate measures to ensure the product meets endophyte specifications therefore avoiding product failure when sown in pastures (Rolston and Agee 2007). Storage of harvested seed at ambient conditions close to points of sale can result in rapid declines in endophyte viability to below this 70% level (Wheatley et al. 2007; Hume et al. 2011). Research has therefore focussed on ways to maintain viable endophyte levels within specifications until seed is sold.

As outlined previously, temperature and humidity are the key environmental parameters that drive the viability of endophyte in stored seed. While specialised controlled condition storage facilities (i.e. low temperature and humidity) can be used for high value commercial seed (nucleus/breeders seed) and valuable genetic resources (grass and fungal) (Rolston et al. 2002; Hume et al. 2011) this significantly increases storage costs for large quantities of seed in commercial production. For these large
quantities, geographic location can be a feasible option for short-term seed storage. For example, in New Zealand, the further south seed is stored the greater the longevity of endophyte, corresponding closely with reduced ambient temperatures and humidity (Hume et al. 2011).

The relative importance of controlling temperature and humidity in storage facilities is a critical issue, particularly as the thermodynamic properties of moist air mean that lowering temperature increases the relative humidity. Effects on endophyte longevity will therefore be positive as a result of a lower temperature but negative as a result of higher relative humidity. These thermodynamic properties of air are not simple linear relationships, with changes dependent on levels of each parameter. Using multiple regression analysis, Welty et al. (1987) in the USA reported linear models to predict endophyte survival from time (length of storage) (0-18 months), temperature (10, 20, 30°C), and seed moisture content (5-24%). For tall fescue, time had the greatest influence, while seed moisture content had more influence than time for perennial ryegrass. For both grasses, seed moisture content had up to a 2-fold greater effect on endophyte survival than temperature. Our interpretation of the data from Welty et al. (1987) is that curvilinear models may provide a better fit and therefore prediction. Increases in endophyte longevity were most responsive when temperature was below 20°C and seed moisture contents kept below 10-12%. At least for seed moisture content, similar effects occur for annual ryegrass (Gundel et al. 2009). In contrast, for perennial ryegrass in New Zealand, Rolston et al. (1994) reported that endophyte longevity was not responsive to seed moisture below 11.5% after 12 months storage but after 24 months this threshold had dropped to 8.5% seed moisture. At ambient conditions the greatest losses in endophyte occur over the summer and early autumn (Hume and Rolston unpublished data). At this time of the year, relative humidity is low compared with winter, so the high temperatures of summer/autumn are likely to be the critical driver of endophyte loss.

In commerce, temperature is relatively cheap to control compared with controlling relative humidity, so research has investigated maintaining seed moisture at low levels through the use of moisture barrier packaging. For perennial ryegrass, aluminium-polyethylene laminated bags can effectively maintain seed at low moisture content and maintain endophyte viability for up to 15 years when combined with cold storage (Rolston et al. 2002). While expensive, such packaging is used on a commercial scale in New Zealand, Rolston et al. (2002) reported that endophyte AR542 (MaxQ®). Polyethylene bags are a lower cost option but are semi-permeable reducing their overall effectiveness, although bags as thick as 140 μm can be effective for up to 4 years at ambient storage conditions (Hare et al. 1990).

Conclusion

For pastoral farmers to capture the beneficial traits of *Neotyphodium* endophytes in temperate grasses, it is crucial that we understand the biological processes involved in endophyte viability within stored seed. Determining the genetic, management, and environmental factors involved, and their relative importance, is critical for the production and distribution of endophyte-infected grasses.

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