Effects of fungicide treatment on transmission of *Epichloë* endophyte in rye (*Secale cereale*)

David E. HUME[1]*, Joanne B. DRUMMOND[2], M. Phil ROLSTON[2], Anouck C. M. de BONTH[1], Paul H. MACLEAN[1], Wayne R. SIMPSON[1], Richard D. JOHNSON[1] and John R. CARADUS[3]

[1]*AgResearch Group – Bioeconomy Science Institute, Palmerston North, New Zealand*

[2]*Foundation for Arable Research, Hornby, Christchurch, New Zealand*

[3]*Grasslanz Technology Ltd., Palmerston North, New Zealand*

*Corresponding author: david.hume@agresearch.co.nz

Abstract

Seed-borne asexual species of *Epichloë* fungal endophyte have proven to be of critical importance in temperate grasses, particularly those grasses sown in pastures in the USA, New Zealand and Australia. These obligate mutualistic fungal endophytes impart benefits to the host plant through enhancing the plant's ability to counter various abiotic and biotic stresses. Although there may be detrimental effects on livestock, selected endophytes have been identified and used to address animal health and productivity issues, while still providing protection to the host grass from stresses.

The use of selected endophytes has also been explored for cultivated cereals such as rye and wheat (*Triticum* spp.), with the goals of reducing usage of fungicides and insecticides, and improving the plants tolerance of abiotic stresses. A complicating factor is that fungicides may still be required for crop management, which in turn could be detrimental to the *Epichloë* endophyte. While this issue has been studied for pasture and amenity grasses, no similar studies have been performed with endophyte-infected cereals. Fungicide treatments, representing a total of five chemical families, were applied either to seed prior to sowing or to foliage of established crops of rye infected with selected strains of *Epichloë bromicola* (AR3002) or *Epichloë bromicola* × *amarillans* (AR3068). In a field trial, two spring applications of five foliar-applied fungicidal products had no detrimental effect on transmission of endophyte to seed (seed-borne endophyte), but one combination did result in 8% lower viable endophyte in the seed. In a greenhouse trial, three seed-applied fungicidal products had no detrimental effect on transmission of endophyte from seed to seedlings. These results demonstrate that if fungicides are required, then options are available that will not adversely affect the *Epichloë* endophyte. In addition to further work on rye, the effect of fungicides on *Epichloë*-infected wheat requires investigation.

Keywords: cereal, *Epichloë bromicola*, *Epichloë bromicola* × *amarillans*, *Triticum*

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) are important temperate grasses that are used worldwide in sown pastures and in turf for amenity areas (Jung et al. 1996; Ball et al. 2019). Much of their success in large areas of grazed pastures in the USA, Australia and New Zealand, is due to their naturally associated asexual *Epichloë* fungal endophytes (Schardl 2010; Leuchtmann et al. 2014). These endophytes are seed-borne, forming enduring obligate mutualistic associations with the grass host. The endophytes bring improved tolerance to a range of biotic and abiotic stresses for the grass. While the resulting agronomic benefits are particularly valued by livestock farmers, some strains of endophyte can cause significant issues with animal health, welfare and productivity (Hume et al. 2016; Ball et al. 2019). For each grass species, selected strains of endophyte have been identified and utilised in elite cultivars, reducing or eliminating the detrimental effects on livestock, while still providing agronomic advantages to the host grass (Caradus & Johnson 2020).

Important cereal grasses such as wheat (*Triticum* spp.), barley (*Hordeum vulgare* L.) and rye (*Secale cereale* L.) do not naturally host *Epichloë* endophytes (Simpson et al. 2014) but through artificial infection, novel synthetic cereal-*Epichloë* associations have been formed (Simpson et al. 2014; Li et al. 2021; Wang et al. 2022; Simpson et al. 2024). This offers the potential to utilise the bio-protective properties of endophytes to address the need for new, natural approaches to control insect pests and plant pathogens

ISSN 2463-2872 (Print) ISSN 2463-2880 (Online) https://doi.org/10.33584/rps.18.2025.3768

in cereal seed production (Simpson et al. 2014). In turn this has the potential to reduce the use of synthetic chemicals, thereby contributing to environmental and sustainability goals of global food production systems (Lamichhane 2017).

Rye-*Epichloë* associations have been successfully formed and genetic compatibility between fungus and host plant improved through recurrent selection to overcome deficiencies in shoot morphology (Simpson et al. 2014; Simpson et al. 2018). Subsequent research has demonstrated the effectiveness of endophyte in rye in reducing some foliar pathogens and pest insects, and increasing plant growth (Hume et al. 2018; Simpson et al. 2018; Popay et al. 2023).

For pasture and amenity grasses some fungicides which are necessary for seed crop management have the potential to adversely affect the viability of *Epichloë* endophytes in the harvested seed (Rolston & Agee 2007). Trialling with tall fescue and ryegrass (*Lolium* spp.) has been conducted in the USA and New Zealand to identify fungicides that are endophyte-safe for use with each grass species (Rolston & Agee 2007). Trials have included fungicides that are applied for control of foliar diseases such as stem rust (*Puccinia graminis* subspecies *graminicola*) (Rolston et al. 2002; Rolston & Agee 2007), seed head diseases such as blind seed (*Gloeotinia temulenta*) (Chynoweth et al. 2012; Sandoval Cruz et al. 2018), and seedling damping-off diseases (Hill & Brown 2000). These trials have sought to investigate the effects of variables such as fungicidal active ingredient, fungicidal product, combinations of active ingredients/products, application rates and dates, and interaction with other inputs such as plant growth regulators (Rolston & Agee 2007). Fungicides that are safe to use on endophyte-infected tall fescue and ryegrass have been identified but may vary depending on each grass-endophyte association (e.g. Hill & Brown 2000; Rolston et al. 2002; Card et al. 2011; Chynoweth et al. 2012; Sandoval Cruz et al. 2018).

Fungicide seed treatments are commonly applied to cereal seeds for control of seed and soil-borne diseases, and fungicides are applied to control foliar diseases on cereal plants as the crop grows. To date, there have been no studies that document the effect these fungicides may have on cereal-*Epichloë* associations. This study investigated the viability and transmission of *Epichloë* in a rye seed crop following the application of fungicides as either a seed coating or when applied to the foliage in spring.

Materials and Methods

The two trials were conducted at the AgResearch farm and glasshouse facilities at Lincoln, New Zealand (-43.627475, 172.470878). The rye-endophyte lines used in this study were sourced from a programme developing cereal-*Epichloë* associations (Simpson et al. 2014). The two endophyte strains used were *Epichloë bromicola* (endophyte strain AR3002) isolated from *Elymus dahuricus* and *Epichloë bromicola* × *amarillans* (endophyte strain AR3068) isolated from *Elymus mutabilis*. Both strains were inoculated into rye cultivar 'Rahu'.

Trial 1: Foliar-applied fungicides

In the 2017-18 growing season, a field trial was conducted to evaluate the effect of foliar fungicide application on endophyte transmission to seed. Six fungicide treatments (Table 1) were applied in late spring to four rye-endophyte lines (Table 2).

Fungicide treatments consisted of three chemical families: strobilurin (as Comet®, emulsifiable concentrate (EC), containing 250 g pyraclostrobin active ingredient (a.i.)/L); triazole (as Opus®, suspension concentrate (SC), containing 125 g epoxiconazole a.i./L, or as Folicur®, water dispersible granule, containing 430 g tebuconzaole a.i./L); pyrazole (as Seguris® Flexi, EC, containing 125 g isopyrazam a.i./L).

Trial management

The trial was sown on 31 May 2017 into a Templeton silt loam of good fertility (e.g. pH_{water} 6.8, Olsen P 25 µg/mL). Plots consisted of single 4 m long rows sown at the equivalent of 130 kg seed/ha. The trial was laid out as a randomised block, split-plot design, with fungicide treatments as main plots and rye-endophyte lines as subplots (6 fungicides × 4 rye-endophyte lines × 6 replicates = 144 plots). The fungicides were applied to the crop at Zadoks Cereal Growth Stage (Zadoks et al. 1974) (GS) 39 (flag leaf fully emerged on the main stem) (13 November 2017) and GS59 (head fully emerged, pre-anthesis) (27 November 2017).

On 7 August 2017, herbicides were applied at 100 g/ha diflufenican a.i. (Quantum® SC) and 1800 g/ha mecoprop a.i., 450 g/ha MCPA a.i. and 56.1 g/ha dicamba a.i. (Trimec® soluble concentrate). Nitrogen was applied at the rates of 50 kg N/ha (as urea) on 12 September and 6 October, and 55 kg N/ha (as ammonium sulphate) on 18 December 2017. Plant growth regulators (750 g/ha chlormequat-chloride a.i. as Cycocel® and 50 g/ha trinexapac-ethyl a.i. as Moddus Evo®) were applied to all plots on 3, 12 and 20 Oct 2017. To reduce the risk of Yellow Dwarf Virus, aphids were controlled with pirimicarb (Pirimor® water dispersible granule) applied at 100 g a.i./ha on 13 November 2017.

Table 1 Foliar fungicides applied to rye-endophyte seed lines (Trial 1).

Fungicide active ingredient(s) (a.i.) and rates applied (a.i. g/ha)	Chemical family (mode of action[1])
Untreated	-
Pyraclostrobin 200	Strobilurin (QoI)
Isopyrazam 125 + pyraclostrobin 200	Pyrazole (SDHI) + Strobilurin (QoI)
Tebuconzaole 189	Triazole (DMI)
Pyraclostrobin 250 + epoxiconazole 125	Strobilurin (QoI) + Triazole (DMI)
Epoxiconazole 125	Triazole (DMI)

[1]QoI, quinone outside inhibitors. SDHI, succinate dehydrogenase inhibitors. DMI, demethylation inhibitors.

Table 2 Seed and endophyte parameters of the four rye-endophyte seed lines used in the foliar-applied fungicide trial (Trial 1). All lines were harvested in the 2016-17 season.

Endophyte status of rye seed line	Endophyte treatment name (description)	TSW (g)[1]	Germination (%)[2]	Viable Endophyte (%)[3]
AR3002	AR3002-14/15 (elite selection made in 2014-15)	22.13	89	98
AR3002	AR3002-15/16 (elite selection made in 2015-16)	23.98	89	93
AR3002	AR3002-16/17 (elite selection made in 2016-17)	23.69	89	95
AR3068	AR3068 (elite selection made in 2016-17)	23.24	90	72

[1]Thousand seed weight (TSW); 500 seeds counted and weighed.
[2]Laboratory test of 100 seeds per line (7-day count).
[3]Determined by tissue print immuno-blot tiller test (Simpson et al. 2012) for 152–290 seedlings per line.

The trial was protected from bird predation using netting. Seed was hand harvested from each plot at GS89 (1 February 2018) and processed using a single plant thresher.

Measurements

On 25 October 2017, on average 43 tillers (at least 27 and up to 70) were sampled per plot and tested for endophyte presence using the tissue print immuno-blot tiller test.

The effect of the fungicides on transmission of endophyte to the harvested seed (termed 'seed-borne endophyte') was determined using a PCR method with high-resolution melt analysis (Gagic et al. 2018) (Slipstream Automation, Palmerston North, New Zealand). This test cannot distinguish between viable and non-viable endophyte, but it can identify, and therefore confirm, endophyte strain. For AR3068, 30 seeds were tested per plot and for the three AR3002 lines 22 seeds were tested per plot.

The harvested seed was then stored at 3°C and 30% relative humidity until 24 July 2018 when it was sown in potting mix in trays in the greenhouse (36 seeds per plot). On 18 September, 8 weeks after sowing, the number of emerged seedlings were counted to determine seed viability (termed 'emergence'). Emerged seedlings were then tested for endophyte presence using the tissue print immuno-blot tiller test (1 tiller tested per seedling) (termed 'endophyte viability').

Weather data

Over the field trial period, June 2017 to January 2018, rainfall was 432 mm (20-year long-term (LT) mean 395 mm), with a mean air temperature of 12.1˚C (LT mean 11.0˚C) (Broadfield weather station H32642 and H32645, NIWA).

Table 3 Fungicide seed treatments applied to rye-endophyte seed lines (Trial 2).

Fungicide active ingredient(s) (a.i.) and rates applied (a.i. g/kg seed)	Chemical family (mode of action)
Untreated	-
Fluxapyroxad 0.416	Pyrazole (SDHI)
Triticonazole 0.040 + prochloraz 0.120	Triazole (DMI) + Imidazole (DMI)
Ipconazole 0.080 + metalaxyl 0.064	Triazole (DMI) + Phenylamide (rRNA inhibitor)

Table 4 Seed and endophyte parameters of the four rye-endophyte seed lines used in the seed-applied fungicide trial (Trial 2).

Endophyte status of rye seed line	Endophyte treatment name (description)	TSW (g)[1]	Emergence (%)	Viable endophyte (%)
AR3002	AR3002 (elite selection made in 2016-17)	16.1	90	85
AR3068	AR3068 (elite selection made in 2018-19)	20.2	80	89
Nil	Nil-1 (AR3002 2016-17 elite selection heat-treated and multiplied, to form an endophyte-free line)	23.7	90	0.5
Nil	Nil-2 (commercially-sourced)	23.5	97	0

[1] Thousand seed weight (TSW); 400 or 800 (AR3068) seeds counted and weighed per line.

Trial 2: Seed-applied fungicides

In this trial, the effect of four fungicide seed treatments (Table 3) on four rye-endophyte seed lines (Table 4) was tested in a factorial design with four replicate blocks (4 fungicides × 4 rye-endophyte lines × 4 replicates = 64 plots). Each block was arranged as a modified Latin square design with rows and columns.

The fungicides were selected based on chemical families for products available at the time on the New Zealand market: pyrazole (as Systiva®, SC, containing 333 g fluxapyroxad a.i./L); triazole (as Kinto® Duo, SC, containing 20 g triticonazole a.i./L, or as Rancona® Dimension, micro-emulsion, containing 25 g ipconazole a.i./L); imidazole (as Kinto® Duo, SC, containing 60 g prochloraz a.i./L); phenylamide (as Rancona® Dimension, micro-emulsion, containing 20 g metalaxyl a.i./L).

The rye seed lines were harvested in the 2018-19 summer at Lincoln (Table 4). From harvest until the trial commenced in September 2019, the seed was stored at 0ºC and 30% relative humidity in the Margot Forde Genebank in Palmerston North, New Zealand. Each seed line was characterised for seed and endophyte traits (Table 4). Seed viability was determined by sowing at least 145 seeds and up to 288 seeds per line in potting mix in trays in the greenhouse and counting the number of emerged seedlings after 4 weeks (‘emergence’). Once the seedlings had formed 2-5 tillers, they were tested for endophyte presence using the tissue print immuno-blot tiller test (1 tiller tested per seedling) (116–258 seedlings tested per line).

Each seed line was surface treated on 3 September 2019 with one of three fungicide products, while the control was left untreated (Table 3). For each seed treatment, 60 seeds per seed line and per replicate were sown in trays filled with potting mix. The trays were placed in a plastic-covered tunnel house with adjustable side ventilation and watered as required while seeds germinated and seedlings developed.

Four weeks after sowing (1-2 October), emerged seedlings were counted and recorded for the proportion of seeds that developed into seedlings (‘emergence’). Seedlings were then tested for endophyte presence using the tissue print immuno-blot tiller test (1 tiller tested per seedling) at GS13-25 (tillering stage).

Statistical analysis

Data from both trials were analysed using linear regression modelling with the factors of endophyte and fungicide, and the interaction between endophyte and fungicide. Each variable was transformed using square root arcsine prior to analysis, with the predicted means

Table 5 Endophyte infection of rye tillers in spring and in harvested seed; meaned across six foliar-applied fungicide treatments grown during the 2017-18 season at Lincoln (Trial 1). Spring tiller endophyte infection was determined prior to application of fungicide treatments. Means followed by the same letter within a column are not significantly different (P≥0.05). SEM = standard error of the mean.

Endophyte treatment name	Spring tiller endophyte infection (%)	Harvested seed	
		Seed-borne endophyte (%)	Viable endophyte (%)
AR3002-14/15	94 a	93 b	92 ab
AR3002-15/16	91 b	92 b	89 b
AR3002-16/17	97 a	98 a	95 a
AR3068	70 c	52 c	35 c
Mean	88	84	78
P-value	<0.001	<0.001	<0.001
SEM	2	3	2

and the lower and upper confidence interval limits back transformed following analysis. The post-hoc tests were performed using the predictmeans package version 1.1.1 (Luo et al. 2024). P-values less than 0.05 (α) were considered statistically significant. All analyses were conducted in R version 4.4.2 (R Core Team 2024).

Results

Trial 1: Foliar-applied fungicides

Spring tiller blots

The endophyte tiller blotting in late October was conducted to establish the rate of endophyte infection in plots prior to the application of fungicides. The overall mean of the spring tiller infection (88%, Table 5) did not differ to the viable endophyte of the sown seed (90%) (Table 2) (P≥0.05). AR3068-infected rye tillers had a lower spring tiller infection level (70%) than the AR3002-infected ryes (all >90%), with some differences between the three AR3002 lines (Table 5).

Harvested seed

There were no effects of fungicide treatment (P=0.406) or rye-endophyte line × fungicide interaction (P=0.174) for seed-borne endophyte. For viable endophyte there was an effect of fungicide (P=0.042) (Table 6) but no rye-endophyte line × fungicide interaction (P=0.896). Pyraclostrobin + epoxiconazole had a lower viable endophyte level than untreated, while all other treatments did not differ from untreated (Table 6).

There were differences between rye-endophyte lines for seed-borne endophyte (P<0.001) and viable endophyte (P<0.001) (Table 5). Seed-borne and viable endophyte levels for AR3002 lines were ≥89% and not different to the spring tiller blots (P≥0.05). In contrast, AR3068 had 70% infected tillers in spring but in the harvested seed only 52% seed-borne endophyte and 35% viable endophyte (P<0.001).

For emergence of the harvested seed, there were no effects of rye-endophyte seed line (P=0.065), fungicide treatment (P=0.056), or rye-endophyte seed line × fungicide interaction (P=0.224) (mean 89%).

Trial 2: Seed-applied fungicides

For seedling emergence, there was an interaction between fungicide treatment and rye-endophyte seed line (P=0.026), due to differing responses of AR3068 compared with AR3002 and Nil endophyte lines. For AR3068-infected rye, all fungicides improved emergence over the untreated seed (P<0.05) (Table 7). For AR3002-infected rye and the two Nil endophyte lines, emergence was high (means of 99 or 100%) and

Table 6 Effects of foliar-applied fungicide treatments on viable endophyte in harvested seed; meaned across four rye-endophyte lines grown during the 2017-18 season at Lincoln (Trial 1). Means followed by the same letter within a column are not significantly different (P≥0.05).

Fungicide active ingredient(s) applied	Viable endophyte (%)
Untreated	83 a
Pyraclostrobin	81 a
Isopyrazam + pyraclostrobin	84 a
Tebuconzaole	84 a
Pyraclostrobin + epoxiconazole	75 b
Epoxiconazole	79 ab
Mean	81
P-value	0.042
SEM	2

unaffected by the addition of fungicides (P≥0.05).

Table 7 Effect of seed-applied fungicides on emergence of the AR3068 endophyte-infected rye seed line (Trial 2). Means followed by the same letter are not significantly different (P≥0.05).

Fungicide active ingredient(s) applied	Emergence (%)
Untreated	86 d
Triticonazole + prochloraz	97 ab
Ipconazole + metalaxyl	94 b
Fluxapyroxad	100 a
P-value	0.026
SEM	3

For viable endophyte, there was an interaction between fungicide treatment and rye-endophyte seed line (P=0.022) (Table 8). For AR3002-infected rye, ipconazole + metalaxyl treated seed had higher viable endophyte than untreated seed (P<0.05), while triticonazole + prochloraz and fluxapyroxad treatments were not different to untreated (P≥0.05). For AR3068, all fungicides were not different to untreated seed (P≥0.05). The two Nil endophyte lines were both free of viable endophyte for all fungicide treatments.

Table 8 Effect of seed-applied fungicides on viable endophyte (%) of two endophyte-infected rye seed lines (Trial 2). Means followed by the same letter are not significantly different at P≥0.05.

Fungicide active ingredient(s) applied	Endophyte strain	
	AR3002	AR3068
Untreated	89 b	80 c
Triticonazole + prochloraz	86 bc	82 bc
Ipconazole + metalaxyl	97 a	86 bc
Fluxapyroxad	83 bc	86 bc
P-value	0.022	
SEM	3	

Discussion

The purpose of this study was to investigate the effects of a range of fungicides on *Epichloë* transmission and viability in rye. In total, seven fungicidal products were tested, covering four modes of action, five chemical families and a total of nine active ingredients. Only in one case was there a negative impact of the applied fungicide, with an effect on endophyte transmission to seed.

For the five fungicides, from three chemical families (pyrazole, strobilurin, triazole) and four active ingredients applied in spring to the rows of endophyte-infected rye, none adversely affected the transmission of endophyte to the harvested rye seed (seed-borne endophyte). In terms of viable endophyte in harvested seed, a combination of pyraclostrobin and epoxiconazole did lower viable endophyte by 8 percentage units relative to untreated plants, despite no effects when applied separately. Epoxiconazole, isopyrazam, pyraclostrobin and tebuconazole have been tested on endophyte-infected ryegrass (strains AR1 and AR37) and tall fescue (strain AR584) seed crops as part of evaluating a range of fungicides that are used in seed production for control of diseases such as blind seed. In ryegrass, none of these fungicides adversely affected viable endophyte in the harvested seed (Rolston et al. 2002; Chynoweth et al. 2012; Sandoval Cruz et al. 2018), while in tall fescue, epoxiconazole and tebuconazole did reduce viable endophyte in the harvested seed (isopyrazam and pyraclostrobin not tested) (Card et al. 2011). With ryegrass, an epoxiconazole-pyraclostrobin mix (and an epoxiconazole-azoxystrobin mix) did reduce viable endophyte transmission (Chynoweth et al. 2012), similar to the result with rye in Trial 1. These results combined, illustrate that responses of *Epichloë* to applied fungicides may vary between grass-endophyte associations and with combinations of fungicides. Additional research is required for all species to determine whether the viability of endophyte during seed storage is influenced by fungicides applied to crops before seed harvest (Rolston et al. 2002; Rolston & Agee 2007).

The seed-applied fungicides tested in this study were representative of four chemical families (imidazole, phenylamide, pyrazole, triazole) and five active ingredients in three formulations available at the time of testing. None of these were detrimental to *Epichloë* viability in the treated seed, when seeds were sown shortly after fungicide application. Other studies have tested various seed-applied fungicides as researchers investigated methods of eliminating endophyte from seeds for purposes of plant breeding, seed production and animal research. Of the active ingredients applied in Trial 2, only triticonazole and prochloraz have been previously tested on endophyte infected seed lots of ryegrass and tall fescue. Both have greatly reduced the incidence of endophyte in plants grown from the treated seeds, however, the application rates were considerably higher than used in our study (Harvey et al. 1982; Latch & Christensen 1982; Leyronas et al. 2006). For prochloraz, Latch and Christensen (1982) achieved approximately 50% 'elimination' of endophyte with the lowest application rate of 0.1 g a.i./kg ryegrass seed, which is similar to the 0.12 g a.i./kg rye rate used in Trial 2 of the current study where there was no effect on endophyte in rye.

Both endophyte strains had a similar response to the foliar and seed-applied fungicides. These strains are genetically distinct and have differing endophyte alkaloid profiles (Card et al. 2014; Popay et al. 2023; Simpson et al. 2024). Further testing of *E. bromicola*-based *Epichloë* strains is needed to determine if endophytes of these species in general are robust to the fungicides that may be applied during seed crop management.

CONCLUSIONS

This study provides a level of confidence that growers will have a range of fungicides to use that will not compromise the *Epichloë* endophyte infection of a rye seed crop. Further testing is needed of rye-endophyte associations, involving more fungicides, and variations in rates, timing of applications and combinations of fungicidal products. Similar work will be required to evaluate the effect of fungicides on *Epichloë*-infected wheats as they are developed (Wood et al. 2024).

ACKNOWLEDGEMENTS

We thank NZ Arable for sowing the field trial. We are grateful for funding from the New Zealand Government (MBIE contracts C10X0807 and C10X1403), Grasslanz Technology Limited (PN23098), Grains Research and Development Corporation (GTL1709-001RTX) and the Foundation for Arable Research.

REFERENCES

Ball DM, Lacefield GD, Hoveland CS. 2019. *The wonder grass: The story of tall fescue in the United States*. Salem, Oregon, USA: Oregon Tall Fescue Commission. https://aurora.auburn.edu/handle/11200/49449

Caradus JR, Johnson LJ. 2020. *Epichloë* fungal endophytes—from a biological curiosity in wild grasses to an essential component of resilient high performing ryegrass and fescue pastures. *Journal of Fungi 6*: 322. https://doi.org/10.3390/jof6040322

Card SD, Rolston MP, Park Z, Cox N, Hume DE. 2011. Fungal endophyte detection in pasture grass seed utilising the infection layer and comparison to other detection techniques. *Seed Science and Technology 39*: 581-592. https://doi.org/10.15258/sst.2011.39.3.05

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 Microbial Ecology 88*: 94-106. https://doi.org/10.1111/1574-6941.12273

Chynoweth RJ, Rolston MP, Kelly M, Grbavac N. 2012. Control of blind seed disease (*Gloeotinia temulenta*) in perennial ryegrass (*Lolium perenne*) seed crops and implications for endophyte transmission. *Agronomy New Zealand 42*: 141-148.

Gagic M, Faville MJ, Zhang W, Forester NT, Rolston MP, Johnson RD, Ganesh S, Koolaard JP, Easton HS, Hudson D, Johnson LJ, Moon CD, Voisey CR. 2018. Seed transmission of *Epichloë* endophytes in *Lolium perenne* is heavily influenced by host genetics. *Frontiers in Plant Science 9*: 1580. https://doi.org/10.3389/fpls.2018.01580

Harvey IC, Fletcher LR, Emms LM. 1982. Effects of several fungicides on the *Lolium* endophyte in ryegrass plants, seeds and in culture. *New Zealand Journal of Agricultural Research 25*: 601-606. https://doi.org/10.1080/00288233.1982.10425226

Hill NS, Brown E. 2000. Endophyte viability in seedling tall fescue treated with fungicides. *Crop Science 40*: 1490-1491. https://doi.org/10.2135/cropsci2000.4051490x

Hume DE, Ryan GD, Gibert A, Helander M, Mirlohi A, Sabzalian MR. 2016. *Epichloë* fungal endophytes for grassland ecosystems. In: Lichtfouse E Ed. *Sustainable Agriculture Reviews*, pp. 233-305. Switzerland: Springer International Publishing. https://doi.org/10.1007/978-3-319-26777-7_6

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: *10th International Symposium on Fungal Endophyte of Grasses*. Salamanca, Spain. Pp. 102. https://digital.csic.es/bitstream/10261/167629/1/Book%20of%20abstracts%20ISFEG10%20Salamanca2018.pdf

Jung GA, Van Wijk AJP, Hunt WF, Watson CE. 1996. Ryegrasses. In: Moser LE, Buxton DR, Casler MD Ed. *Cool-Season Forage Grasses*, pp. 605-641. Madison, Wisconsin: ASA, CSSA and SSSA. https://doi.org/10.2134/agronmonogr34.c19

Lamichhane JR. 2017. Pesticide use and risk reduction in European farming systems with IPM: An introduction to the special issue. *Crop Protection 97*: 1-6. https://doi.org/10.1016/j.cropro.2017.01.017

Latch GCM, Christensen MJ. 1982. Ryegrass endophyte, incidence, and control. *New Zealand Journal of Agricultural Research 25*: 443-448. https://doi.org/10.1080/00288233.1982.10417910

Leuchtmann A, Bacon CW, Schardl CL, White Jr JF, Tadych M. 2014. Nomenclatural realignment of *Neotyphodium* species with genus

Epichloë. *Mycologia 106*: 202-215. https://doi.org/10.3852/106.2.202

Leyronas C, Mériaux B, Raynal G. 2006. Chemical control of *Neotyphodium* spp. endophytes in perennial ryegrass and tall fescue seeds. *Crop Science 46*: 98-104. https://doi.org/10.2135/cropsci2005.0135

Li C, Wang Z, Chen T, Nan Z. 2021. Creation of novel barley germplasm using an *Epichloë* endophyte. *Chinese Science Bulletin 66*: 2608-2617. https://doi.org/10.1360/TB-2020-1587

Luo D, Ganesh S, Koolaard J. 2024. *Predictmeans: Predicted means for linear and semiparametric models. R package version 1.1.1*. https://cran.r-project.org/package=predictmeans

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

R Core Team. 2024. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rolston MP, Archie WJ, Simpson WR. 2002. Tolerance of AR1 *Neotyphodium* endophyte to fungicides used in perennial ryegrass seed production. *New Zealand Plant Protection 55*: 322-326. https://doi.org/10.30843/nzpp.2002.55.3899

Rolston MP, Agee C. 2007. Delivering quality seed to specification - the USA and NZ novel endophyte experience. Presented at: *Proceedings of the 6th International Symposium on Fungal Endophytes of Grasses. Grassland Research and Practice Series No. 13*. Christchurch, New Zealand. Pp. 229-231. https://doi.org/10.33584/rps.13.2006.3065

Sandoval Cruz EA, McGill CR, Southward RC, McKenzie CM, Card SD, He XZ, Wang M, Rolston MP, Chynoweth RJ. 2018. Does chemical control of blind seed disease (*Gloeotinia temulenta*) affect endophyte transmission in ryegrass seed crops? *Australasian Plant Pathology 47*: 561-569. https://doi.org/10.1007/s13313-018-0598-0

Schardl CL. 2010. The epichloae, symbionts of the grass subfamily Poideae. *Annals of the Missouri Botanical Garden 97*: 646-665. https://doi.org/10.3417/2009144

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, Popay AJ, Mace WJ, Hume DE, Johnson RD. 2018. Creating synthetic symbioses between *Epichloë* and rye (*Secale cereale*) to improve crop performance. Presented at: *International Symposium on Fungal Endophyte of Grasses*. Salamanca, Spain. Pp. 96. https://digital.csic.es/bitstream/10261/167629/1/Book%20of%20abstracts%20ISFEG10%20Salamanca2018.pdf

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

Wood L, Simpson W, Johnson R, Roy S, Hoffman R, Caradus JR. 2024. Breeding to improve the wheat phenotype while maintaining *Epichloë* endophyte compatibility. Presented at: *3rd International Wheat Congress*. Perth, Australia. Pp. 84-85. https://www.iwc2024.com/wp-content/uploads/2024/10/241008_IWC-Abstract-Book-Workshop-Presentations.pdf

Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. *Weed Research 14*: 415-421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x