

Control of blind seed disease (*Gloeotinia temulenta*) in perennial ryegrass (*Lolium perenne*) seed crops and implications for endophyte transmission

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Abstract

In three field trials fungicides used for stem rust (*Puccinia graminis*) control were evaluated to assess the effect on levels of blind seed disease, seed germination and endophyte (*Neotyphodium lolii*) transmission. Blind seed disease infection was reduced from 24% to 5% in one of the three trials undertaken and germination was increased by between 17 and 23% in two trials. There was a negative linear correlation between germination percentage and blind seed disease percentage ($R^2=0.57$). Endophyte transmission was either reduced or unchanged with fungicide treatment. While the highest level of germination were achieved when Proline fungicide was used; the optimum balance between germination, blind seed and AR37 endophyte transmission was from using Folicur-based demethylation inhibitor fungicide programmes.

Additional keywords: germination, AR37, fungicide

Introduction

Blind seed disease of perennial ryegrass (*Lolium perenne* L.) seed crops has been an issue for New Zealand seed producers since the early 1920s when growers in areas with higher summer humidity, e.g. Southland and Manawatu, were troubled by low seed germinations. Neill and Hyde (1939) identified that the disease is caused by a fungus (*Gloeotinia temulenta*) that invades ryegrass and tall fescue florets during flowering and kills the developing embryo. The infected seed looks normal but is dead. Germinations of as low as 1% have been reported in New Zealand (Greenall, 1943), 13% in the United States (Alderman, 2001) and 50% in Great Britain (Noble and Gray,

1945). Blind seed continues to affect New Zealand seed crops periodically. The life cycle of blind seed disease has been described by Falloon and Rolston (1996). In New Zealand blind seed disease occurs in epidemics and is associated with wet weather conditions during anthesis and early seed fill (Hampton and Scott, 1980). Endophytic fungi (*Neotyphodium lolii*) are also seed borne. Maintaining a high level of endophyte transmission to seed and the subsequent ryegrass pasture is important for protection from Argentine stem weevil and other insect pests. The application of fungicides has been implicated in reduced endophyte transmission.

Economic loss from blind seed epidemics is considerable as significant discounting of seed prices commonly occurs for seed lots that are below 90% germination. Seed with germination below 80% is difficult to retail and may not be saleable in years of good production.

Triazole (DeMethylation Inhibitor, DMI) fungicides are used on perennial ryegrass seed crops to control stem rust (*Puccinia graminis*) infection (Rolston *et al.*, 2009). This paper reports on three trials testing the hypothesis that DMI fungicides used for stem rust control can reduce blind seed infection. The effect of these fungicides on endophyte transmission and/or viability was also assessed.

Materials and Methods

Two of the three trials were undertaken in paddocks of three or four year old perennial ryegrass pasture at the 'AgResearch Farm', Lincoln (43° 38' 09" S, 172° 28' 11" E). Both sites were located where blind seed disease had been detected in the previous season and where substantial seed drop had occurred. To maximise the likelihood of blind seed infection, no spring N was applied (Hampton and Scott, 1981, de Filippi *et al.*, 1996). The plant growth regulator, Moddus (a.i. 250 g/l trinexapac ethyl) was applied at either 1.5 or 2.0 l/ha to create a short, open crop of low dry matter to encourage primary infection from apothecia at the soil surface.

Trial one included eight fungicide treatments with four replicates in a randomised block design. Fungicides consisted of combinations and sequences of Amistar (a.i. 250 g/l azoxystrobin), Protek (a.i. 500 g/l carbendazim), Folicur (a.i. 430 g/l tebuconazole), Opus (a.i. 125 g/l epoxiconazole), Proline (a.i. 250 g/l prothioconazole), Twist (a.i. 500 g/l

trifloxystrobin) and an untreated control (Table 1). Plots were 4 × 4 m with treatments applied at early anthesis (1 December 2005) and late anthesis (12 December 2005). The cultivar was 'Grasslands Samson'. For germination analysis, 400 seeds were pre-chilled (5°C) with 0.2% KNO₃ for 5 days and then placed in a 20°C germinator. Final germination counts (normal seedlings) were based on 14 day assessment as per the International Rules for Seed Testing (ISTA, 2006). Blind seed analysis was carried out on 18 individual plots representing a range of germination results.

Trial two included seven fungicides with three replicates in a randomised block design. The fungicides used were: Amistar; Folicur, Proline and Protek (Table 2). Plots were 4 × 4 m. Treatments were applied at early flowering (9 December 2007) and mid-seed fill (24 December 2007). The cultivar was 'Grasslands Samson' and contained the AR37 Endophyte. Germination analysis consisted of 100 seeds per plot planted in seed raising mix and then raised in a glasshouse.

In trials one and two, harvest consisted of hand cutting one square metre from the centre of each plot, placing plant material in hessian sacks and allowing it to air dry outdoors. Samples were then hand rubbed to remove seed for further processing.

Trial three was located at Plant and Food Research, Lincoln (43° 38' 18" S, 172° 28' 30" E) and used a first year seed crop of cultivar 'Kamo', which contained the AR37 endophyte. The fungicides used were: Amistar; Comet (a.i. 250 g/l pyraclostrobin), Fandango (a.i. 100 g/l fluoxastrobin and 100 g/l prothioconazole), Folicur, Opus, Proline and Protek (Table 3). Plots were 12 by 2 m with treatments applied 5 December 2005, 14 December

2005 and 20 December 2005. At harvest plots were windrowed (14 January 2006) and a hand sample was collected for germination and endophyte testing. Plots were machine harvested using a Wintersteiger plot combine on 28 January 2006. Germination and endophyte analysis consisted of 100 seeds per plot planted in seed raising mix and then raised in a plastic covered tunnel house.

In all trials, blind seed incidence was determined at theASUREQuality Limited seed pathology laboratory (Lincoln, Canterbury) using microscopic examination of seeds that had been soaked and then peeled to expose the caryopsis (Matthew, 1980). Viable endophyte was determined by cutting 2 tillers per plant from 100 plants and using the immunoblot technique (Gwinn *et al.*, 1991).

Data analysis

All data was tested by analysis of variance (ANOVA) and where significant effects were observed ($P < 0.05$), differences were compared using the least significant difference (LSD) procedure ($P = 0.05$). All relationships were tested by linear regression. All data analysis was done using

GenStat (version 9, VSN International Ltd, UK).

Results

Seed germination

In two of the three trials fungicide treatment resulted in an increase ($P < 0.05$) in seed germination compared with the control treatment without fungicide.

Trial one

All fungicide treatments increased germination percentage compared with the untreated control (Table 1). Proline (T8) and Proline followed by Protek (T7) gave the highest germinations (82 and 84%, respectively), although results from treatments with Opus + Amistar + Protek (T4) at anthesis (80%) and Opus followed by Protek (T6) (78%) were not significantly different ($P < 0.05$). Folicur + Protek (T2) (76%) at anthesis was significantly less than Proline followed by Protek (T7), but was similar ($P < 0.05$) to the other fungicides. Low germination was associated with increasing blind seed disease incidence (Figure 1).

Table 4: Germination percentage for eight fungicide treatments applied to perennial ryegrass at early anthesis (1 December) and late anthesis (12 December), cultivar Samson 2005/06, Lincoln (Trial 1).

Treatment No.	Product application rate and timing		Germination (%)
	1 December 2005	12 December 2005	
T1	nil	nil	61
T2	440 ml/ha Folicur + 500 ml/ha Protek	500 ml/ha Protek	76
T3	220 ml/ha Folicur + 500 ml/ha Amistar + 500 ml/ha Protek	500 ml/ha Protek	74
T4	500 ml/ha Opus + 500 ml/ha Amistar + 500 ml/ha Protek	500 ml/ha Protek	80
T5	220 ml/ha Folicur + 500 ml/ha Twist + 500 ml/ha Protek	500 ml/ha Protek	73
T6	1000 ml/ha Opus	500 ml/ha Protek	78
T7	800 ml/ha Proline	500 ml/ha Protek	84
T8	800 ml/ha Proline	nil	82
LSD _(0.05)			7

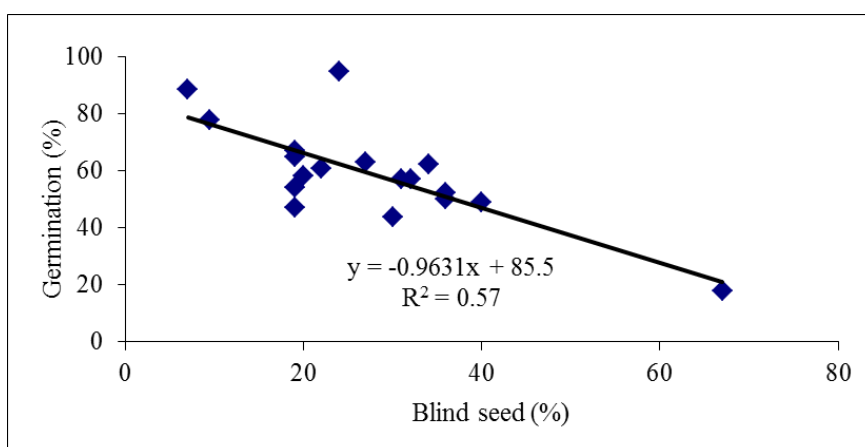


Figure 1: Relationship between blind seed disease infection and germination cultivar Samson 2005-06, Lincoln (Trial 1).

Trial two

Germination (68%) for the nil fungicide control was lower ($P < 0.05$) than all fungicide treatments which resulted in final germinations between 79 and 94%. The fungicide treatments giving the highest germinations contained Proline at anthesis followed by either Proline + Protek (T3 and T6) or Folicur + Protek (T4) at mid-seed fill. These treatments resulted in germinations of between 87 and 94%. There was a strong negative correlation ($R^2 = 0.99$) between blind seed disease infection and germination.

The untreated control had 24% blind seed disease infection (Table 2). All fungicide treatments reduced blind seed infection and increased germination to above 80% except where no fungicide was applied at anthesis. Delaying fungicide application to mid-seed fill (T2) did not improve blind seed control but was enough to improve germination

($P < 0.05$) compared with the control (associated with the increased within treatment variation of blind seed disease, i.e. coefficient of variation = 30%).

Trial two and three contained the AR37 endophyte (*Neotyphodium lolii*). In general the addition of fungicide reduced endophyte transmission, however results were highly variable e.g. coefficient of variation (CV) in trial 3 was 22%. In trial two, 300 ml/ha Folicur + 500 ml/ha Amistar applied at mid-anthesis and mid-seed fill gave the highest endophyte level (86%) which was significantly higher than all other fungicide treatment except control and nil at mid-anthesis followed by 400 ml/ha Proline + 500 ml/ha Protek mid-seed fill. The control endophyte level (78%) was the same as all other treatments ($P < 0.05$) except 400 ml/ha Proline + 500 ml/ha Amistar followed by 400 ml/ha Proline + 500 ml/ha Protek (50% endophyte infection) (Table 2.).

Table 2: Germination, blind seed and endophyte percentages for seven fungicide treatments applied to perennial ryegrass at mid-anthesis (9 December 2007) and mid-seed fill (24 December 2007), cultivar 'Grasslands Samson' 2007-08, Lincoln (Trial 2).

Treatment No.	Product application rate and timing		Germination (%)	Blind seed disease (%)	Endophyte (%)
	9 December 2007	24 December 2007			
T1	nil	nil	68	24	78
T2	nil	400 ml/ha Proline + 500 ml/ha Protek	79	18	72
T3	400 ml/ha Proline	400 ml/ha Proline + 500 ml/ha Protek	94	5	70
T4	600 ml/ha Proline	300 ml/ha Folicur + 500 ml/ha Protek	87	12	64
T5	300 ml/ha Folicur + 500 ml/ha Amistar	300 ml/ha Folicur + 500 ml/ha Protek	82	16	86
T6	400 ml/ha Proline + 500 ml/ha Amistar	400 ml/ha Proline + 500 ml/ha Protek	91	7	50
T7	440 ml/ha Folicur+ 500 ml/ha Amistar	300 ml/ha Folicur + 500 ml/ha Protek	90	11	69
LSD _(0.05)			5	7	15
F-value probability			<0.001	0.002	0.01
CV %			5	30	12

Trial three

In trial three only the 440 ml/ha Folicur + 2000 ml/ha Protek fungicide treatment improved germination. The 2006 harvest was damp with prolonged period of high humidity (data not presented). Germination declined ($P < 0.05$) between windrowing and harvest, 14 days later (75 versus 59% respectively). In 2006 poor germination in ryegrass seed lines was a result of blind seed disease (N. Grbavac, AsureQuality, pers. comm. 2006).

Plants in trial three also contained the AR37 endophyte (*Neotyphodium lolii*). In general the addition of fungicide reduced endophyte transmission, however results

were highly variable e.g. coefficient of variation was 22%. In trial three, endophyte transmission was poor for all fungicide treatments except Folicur (440ml/ha) + Protek (2000 ml/ha) which resulted in 85% transmission compared with that of the untreated control (71%) at harvest (Table 3). Treatments 7 and 8 reduced endophyte levels at harvest but for the other fungicide treatments endophyte levels were not statistically different from the control. However, as in trial two high rates of DMI fungicides repeated during anthesis and seed filling resulted in a trend towards reduced endophyte transmission at harvest.

Table 5: Percentage seedling endophyte at windrowing (hand sampled) and at harvest for 10 fungicide treatments, cultivar Kamo, 2005-06 (Trial 3).

Treatment No.	Product application rate (repeated 3 times ¹)	Hand sampled endophyte (%)	Endophyte at harvest (%)
T1	Nil	83	71
T2	440 ml/ha Folicur	78	66
T3	1000 ml/ha Opus	82	62
T4	2000 ml/ha Protek	89	64
T5	800 ml/ha Proline	61	61
T6	1500 ml/ha Fandango	67	54
T7	600 ml/ha Opus + 800 ml/ha Comet	83	44
T8	1000 ml/ha Opus 750 ml/ha+ Amistar	89	49
T9	440 ml/ha Folicur + 750 ml/ha Amistar	88	69
T10	440 ml/ha Folicur + 2000 ml/ha Protek	85	85
Mean		80	63
LSD _(0.05)		12.8	20
LSD _(0.10)		10.6	16.7
CV%		11.5	22

¹Application dates: 5 December 2005, 14 December 2005 and 20 December 2005.

Discussion

In this study the application of DeMethylation Inhibitor (DMI) fungicides, e.g. Proline, at anthesis was an effective tool for controlling blind seed disease when applied twice (Table 2). In trial one the application of DMI fungicides improved

germination (Table 1), which was correlated with blind seed infection (Figure 1). There was little difference between individual DMI products but there was some evidence of a trend for treatments including Proline to give higher germination levels and reduced blind seed infection. However,

results from Proline treatments were not different ($P < 0.05$) from Folicur or Opus treatments. The follow-up use of Methyl Benzimidazole Carbamates (MBC) e.g. Protek is common practice among ryegrass seed producers for the control of the secondary spread phase of blind seed disease. While Protek was included in this study no data was generated for MBC fungicides alone. The data in trial 3 highlights that windrowing does not mean that the crop is safe from further seed quality loss. In trial three both germination and endophyte were dramatically reduced during the 14 days of exposure to unfavourable climatic conditions. Therefore growers should aim to harvest seed crops at the first possible opportunity and apply fungicides as late as withholding regulations allow, especially when damp environmental conditions prevail.

Some DMI fungicides, e.g. Opus and Proline can have negative effects on endophyte transmission (Table 2 and Table 3); a result previously reported in tall fescue by Rolston and Agee (2006). Some fungicides which have good stem rust and blind seed disease activity are detrimental to AR37 endophyte transmission (unpublished data from authors). Previous work on AR1 endophyte has shown no negative effects from fungicide application (both DMI and strobilurin groups) on endophyte transmission (Rolston *et al.*, 2002), suggesting that each individual endophyte strain may respond differently. This raises a new level of complexity to seed companies and producers.

Conclusions

Stem rust fungicides were effective in reducing the incidence of blind seed disease when applied twice at timings that should not compromise stem rust control.

The best fungicide programmes investigated reduced the level of blind seed disease from $>20\%$ to as low as 5% and germination percentage was increased by between 19 and 24%. In all three trials final seed germination of nil fungicide treatments would have resulted in significant price discounts, whereas in two of the three trials the best treatments gave germinations of $>80\%$.

Neotyphodium endophyte AR37 transmission into germinating seedlings was reduced by two different DMI fungicides in separate trials, suggesting growers need to be cautious when applying these fungicides to seed crops containing the AR37 endophyte.

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