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Control of net form of net blotch in barley from seed- and foliar-applied fungicides

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Abstract. Net form of net blotch (NFNB), caused by *Pyrenophora teres* f. *teres*, is a major foliar disease of barley (*Hordeum vulgare* L.) worldwide that can cause grain yield and quality loss in susceptible varieties. Seed- and foliar-applied fungicides were evaluated in six field experiments infected with NFNB during 5 years, for suppression of NFNB severity and protection of grain yield and quality. Suppression of NFNB severity varied between treatments and experiments. Grain yield and quality improvements were recorded in two experiments. Foliar fungicide applications at stem elongation (Zadoks growth stage Z31) and flag leaf emergence (Z39) or ear emergence (Z55) significantly reduced NFNB severity, increased grain yield by up to 23%, and improved grain-quality measurements of retention, screenings and weight. The seed-applied fungicide fluxapyroxad provided significant reductions in NFNB severity, improvements in grain yield of up to 20%, and improved grain quality. Where NFNB was severe, none of the seed or foliar fungicide application strategies provided complete control of NFNB, indicating that more than two applications were necessary when conditions were favourable for disease development in susceptible varieties.

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Introduction

Net form of net blotch (NFNB), caused by *Pyrenophora teres* f. *teres* (anamorph *Drechslera teres* (Sacc.) Shoem.), is a damaging stubble- and seed-borne foliar disease of barley (*Hordeum vulgare* L.) worldwide (Smedegård-Petersen 1974; Steffenson 1997). Losses of grain yield and quality have been reported in Australia (Khan 1987), Canada (Sutton and Steele 1983; Martin 1985; Martin and Sanderson 1988; Entz *et al*. 1990; Turkington *et al*. 2012), Denmark (Smedegård-Petersen 1974), Estonia (Kangor *et al*. 2017), Germany (Deimel and Hoffmann 1991) and Morocco (Burleigh *et al*. 1988; Yousfi and Ezzahiri 2001, 2002; Jebbouj and Yousfi 2009). Reductions in grain-quality parameters such as grain plumpness, weight and protein have also been reported (Jordan 1981; Khan 1987; Martin and Sanderson 1988). Net form of net blotch is prevalent in most barley-growing regions worldwide where susceptible varieties are cultivated in close rotation (Steffenson 1997), and was found to be greatest in crops sown directly into infected barley residue (Jordan and Allen 1984). This provides challenges to barley growers where time between barley crops is not sufficient to allow breakdown of infected stubble. The greatest losses occur in high yielding barley crops (Karamanos *et al*. 2012), where the top three leaves are affected (Khan 1987). In addition, NFNB can be seed-borne (Steffenson 1997), which means that infection can also occur where infected barley stubble is not present.

Barley varieties resistant to NFNB have been developed in many countries to minimise losses. However, *P. teres* f. *teres* is genetically diverse and can overcome host-plant resistance (Wallwork *et al*. 2016; Akhavan *et al*. 2017; Fowler *et al*. 2017). This has been observed on multiple occasions worldwide following regional cultivation of barley cultivars with resistance and the evolution of virulent *P. teres* f. *teres* pathotypes (Khan 1982; Fowler *et al*. 2017).

Barley growers need information on effective NFNB control strategies to avoid losses where susceptible varieties are grown or resistance has been overcome. Fungicides can provide effective control of NFNB and prevent losses of grain yield and quality. Seed-applied fungicides have been shown to be effective in reducing seed-borne NFNB infection (Martin 1985), and foliar fungicides effective in controlling leaf infection (Turkington *et al*. 2012; Stepanović *et al*. 2016). NFNB can develop rapidly and requires multiple foliar fungicide applications for effective control. Previous studies showed that two foliar applications could be effective, whereas single applications generally provide partial grain-yield and quality benefits (Sutton and Steele 1983; Martin and Sanderson 1988; Stepanović *et al*. 2016). In addition, efficacy of foliar fungicide varied depending on the environment and seasonal conditions (Martin and Sanderson 1988; Entz *et al*. 1990). Most studies have been conducted in the Northern Hemisphere during summer growing seasons. Effective fungicide-application strategies are also needed for winter growing-season environments.

The new systemic seed-applied fungicide fluxapyroxad (Systiva; BASF Australia, Melbourne) provides a novel option for NFNB management because it can provide suppression during the seedling and tillering stages of crop development. It has been shown to be effective in supressing barley scald caused by *Rhynchosporium commune* ((Zaffarano, McDonald & Linde sp. nov.) and providing improvements in grain yield and quality (McLean and Hollaway 2018). No information is available on the effectiveness of fluxapyroxad for NFNB management; and it needs to be evaluated for recommendations to be made.

This study aimed to estimate the level of NFNB suppression and improvements in grain yield and quality provided by: (*i*) one, two or three applications of foliar fungicide (prothioconazole + tebuconazole); (*ii*) different timings of one and two foliar applications in relation to crop growth stage; and (*iii*) seed application of fluxapyroxad. The study also aimed to correlate NFNB severity to grain yield and quality loss.

Methods

Six field experiments were conducted during 2012–16. Experiments were sown at Vectis (E142°11.712′, S36°73.947′), 7 km west of Horsham, or Wonwondah (E142°08.688′, S36°51.627′), 22 km south of Horsham, Victoria, Australia. The sites were characterised by Grey Vertosol clay soils with long-term average growing-season rainfall (May–November) ~315 mm and annual rainfall 450 mm.

Experiment design and treatments

The barley breeding line VB9613 was cultivated in all experiments because it was susceptible to NFNB and resistant to other regionally important foliar diseases such as spot form of net blotch (caused by *P. teres* f. *maculata* (*Drechslera teres* f. *maculata* Smedeg.)), scald (caused by *R. commune*), leaf rust (caused by *Puccinia hordei* Otth) and powdery mildew (caused by *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal). During 2012–13, plots were ~8.0 m long and 1.5 m wide with six rows. During 2014–16, a different seeder was used, and plots were 8.0 m long and 1.8 m wide with five rows. Wheat buffer plots, the same size as test plots, were sown parallel to each barley plot to reduce interplot interference effects due to disease. Plots were sown at ~150 seeds/m^2 with urea and mono-ammonium phosphate fertiliser applied before, or at, sowing as required to obtain grain yield ~5 t/ha. Selective herbicides were applied according to recommendations for the local area to control grass and broadleaf weeds. Barley stubble (~1 kg) naturally infected with *P. teres* f. *teres* was applied to each barley plot 2–4 weeks after sowing to promote development of NFNB.

Six replicates of eight treatments were arranged in complete randomised block design. Treatments are listed in Table 1. Fungicide treatments consisted of seed-applied fluxapyroxad (Systiva), or one, two or three applications of foliar-applied prothioconazole + tebuconazole (Prosaro; Bayer CropScience) at 150 mL/ha (2015) or 300 mL/ha (2012–14). Depending on the treatment, foliar fungicides were applied at Zadoks crop growth development stages Z25 (mid tillering), Z31 (stem elongation), Z39 (flag leaf emergence) and Z55 (ear emergence) (Zadoks *et al*. 1974): one application at Z31, Z39 or Z55; applications at Z31 + Z39 or Z39 + Z55; applications at Z25 + Z31 + Z55. The treatment consisting of three applications was not applied at Vectis during 2016. An untreated control was used to measure maximum disease severity and grain yield and quality loss. Seed used for foliar and control treatments had triticonazole (25 g/L) applied at 150 mL/100 kg seed to control seed-borne loose (*Ustilago nuda*) and covered (*U. hordei*) smut diseases.

Disease severity, grain yield and quality

Disease severity was visually estimated by assessing percentage leaf area affected (%LAA). Estimates were done on the flag to flag-2 leaves at ear emergence–flowering and grainfill stages. During 2016, two additional estimates were done: a whole-plot assessment at Z31 (early stem elongation), and the top three leaves at Z33 (mid stem elongation). Leaves that had not fully emerged or had senesced were not assessed.

Grain was harvested at the end of the season by using a plot harvester, and grain yield measured for each plot. Subsamples were retained from each plot for grain-quality testing of screenings (percentage of grain <2.2 mm in width), retention (percentage of grain >2.5 mm in width), grain weight (1000 grains), and percentage protein (assessed using near infrared (NIR) analysis during 2013–16). Grain yield and quality data

Table 1. Treatments tested to determine suppression of net form of net blotch and improvements to grain yield and quality of barley breeding line VB9613 at Vectis and Wonwondah during 2012–16

Growth stages: Z25, mid tillering; Z31, stem elongation; Z39, flag leaf emergence; Z55, mid ear emergence according to Zadoks *et al*. (1974). Prosaro active ingredients are prothioconazole + tebuconazole (210 + 210 g/L); Systiva active ingredient is fluxapyroxad (333 g/L)

Treatment no.	Treatment description	Product applied	Product rate 2012–14	Product rate 2015
T1	Foliar at Z25 + Z31 + Z55	Prosaro	300 mL/ha	150 mL/ha
T2	Seed	Systiva	150 mL/100 kg	
T3	Foliar at Z39 + Z55	Prosaro	300 mL/ha	150 mL/ha
T4	Foliar at Z31 + Z39	Prosaro	300 mL/ha	150 mL/ha
T5	Foliar at Z39	Prosaro	300 mL/ha	150 mL/ha
T6	Foliar at Z55	Prosaro	300 mL/ha	150 mL/ha
T7	Foliar at Z31	Prosaro	300 mL/ha	150 mL/ha
T8	Untreated control	–	–	–

are not presented for the experiment at Vectis 2015 as it was confounded by scald infection.

Statistical analyses

Data from NFNB severity, grain yield and quality measurements of percentage retention, screenings, protein and 1000 grain-weight were subjected to analysis of variance and Fisher's unprotected least significant difference test at $P = 0.05$ and 0.1. Correlations between NFNB severity on the flag to flag-2 leaves and grain yield and quality were estimated by using simple linear regression analysis and significance was tested by one-sided *t*-test at $P = 0.05$. All statistical tests were done using GENSTAT 14th Edition (VSN International, Hemel Hempstead, UK).

Results

NFNB severity

Severity of NFNB varied between experiments and between treatments within experiments (Table 2). The foliar fungicide treatments with three applications (T1) and with two applications (T3 and T4) provided significant suppression compared with the untreated control (T8) in all experiments. The treatments with seed-applied fungicide (T2) and with a single application of foliar fungicide at Z31 (T7) or Z39 (T5) provided significant suppression compared with the untreated control (T8) in all experiments; however, suppression was not as strong as found for T1, T3 and T4 foliar fungicide treatments in some cases. A single foliar fungicide application at Z55 (T6) provided significant suppression compared with the untreated control (T8) in two experiments.

At Vectis during 2016, seed-applied fluxapyroxad (T2) provided NFNB control at Z31, after which NFNB severity increased; final severity was significantly lower than other treatments except T3 (foliar-applied at Z39 + Z55) (Fig. 1, Table 2). The foliar fungicide treatments all had ~35% LAA at Z31, compared with 2% for T2 (seed-applied). Disease progress varied between foliar fungicide treatments in relation to application timing.

Grain yield

All fungicide treatments provided significant grain yield improvements compared to the untreated control (T8) in experiments at Vectis during 2013 and 2016 (Table 3), where NFNB severity at ripening was 29–93% LAA. There were no significant improvements in grain yield in the other four experiments (data not shown), where NFNB severity was 4–27% LAA at ripening. Grain yield and quality improvements varied between treatments and experiments. During 2013, the foliar fungicide treatments with two applications (T4) and three applications (T1) provided the greatest yield improvements compared to the untreated control (T8), with 15% and 13% improvement, respectively. Applications of fungicide at Z39 + Z55 (T3), and at Z31 (T7) or Z39 (T5), also significantly increased grain yield, by 10%, compared with the untreated control. Three foliar fungicide applications (T1) did not provide significant yield improvements compared to these treatments (5.2 vs 5.0 t/ha, l.s.d. ($P = 0.05$) = 0.23). During 2016, seed application treatment (T2) and treatments with two foliar applications (T3 and T4) of fungicide provided the greatest yield improvements compared to the untreated control (T8). Of the single foliar fungicide application treatments, treatment at Z39 (T5) was most effective during both seasons, with treatment at Z31 (T7) comparable to treatment at Z39 (T5) in one experiment, whereas treatment at Z55 (T6) was less effective than T5 in both experiments.

Grain quality

Fungicide treatments provided significant improvements in grain quality measurements of percentage retention and screenings in two experiments and 1000-grain weight in one experiment (Table 3). No improvements in grain protein were recorded in any experiment (data not shown). During 2013, the seed (T2), single foliar at Z39 (T5) and two foliar (T3 and T4) application treatments and three foliar application treatment (T1) provided significant improvements to grain retention and screenings

Table 2. Net form of net blotch severity (% leaf area affected) on barley breeding line VB9613 at grain ripening in response to seed and foliar fungicide application at different timings at Vectis and Wonwondah during 2012–16

GSR, Growing season rainfall, April–November (long-term average 315 mm). Foliar treatments are Prosaro (a.i.s prothioconazole + tebuconazole at 210 + 210 g/L, applied at 300 mL/ha during 2012–14 and 150 mL/ha 2015–16). Seed treatment is Systiva (a.i. fluxapyroxad at 333 g/L, applied at 150 mL/100 kg seed). Growth stages: Z25, mid tillering; Z31, stem elongation; Z39, flag leaf emergence; Z55, mid ear emergence (Zadoks *et al*. 1974). –, Treatment not applied. Within columns, means followed by the same letter not significantly different at $P = 0.05$

Treatment	2012 260 mm		2013 356 mm	2014 213 mm	2015 142 mm	2016 361 mm
GSR:	Vectis	Wonwondah	Vectis	Vectis	Vectis	Vectis
T1. Foliar Z25 + Z31 + Z55	0.6a	0.0a	0.9a	3.6ab	1.6ab	–
T2. Seed	2.4b	1.3b	1.8a	15.0c	4.0c	42.1b
T3. Foliar Z39 + Z55	3.4bc	2.1b	0.6a	2.5a	1.3ab	28.8a
T4. Foliar Z31 + Z39	0.4a	0.1a	1.4a	0.7a	0.6a	53.8c
T5. Foliar Z39	3.5c	1.5b	1.1a	2.7a	1.2ab	59.0d
T6. Foliar Z55	5.8d	3.6c	25.3c	13.9c	13.8e	67.3e
T7. Foliar Z31	0.9a	1.5b	14.7b	5.9b	2.7bc	86.5f
T8. Untreated control	4.9d	4.0c	28.5c	26.5d	11.3d	92.6 g
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
l.s.d. ($P = 0.05$)	1.22	0.973	4.024	3.59	1.74	5.83

compared with the untreated control (T8). Single foliar fungicide application at Z31 (T7) did not provide grain quality improvements compared to the untreated control. During 2016, foliar fungicide application at Z55 (T6) provided significant improvement in grain retention and screenings, but not during 2013. All other treatments showed significant improvements for retention, screenings and 1000-grain weight, with application of foliar fungicide at Z39 + Z55 (T3) providing the greatest improvements in grain quality.

Correlations

Correlations between NFNB severity on the top three leaves and grain yield and quality varied between the 2013 and 2016 experiments (Table 4). During 2013, grain yield and quality were similarly correlated with NFNB severity on the top three leaves. During 2016, grain yield and quality were correlated with disease severity on the flag and flag-1 leaves, but not flag-2. There was a significant negative relationship between NFNB severity on flag-1 at grain ripening and grain yield in both experiments (Fig. 2).

Discussion

Net form of net blotch was severe and caused up to 23% (1.2 t/ha) grain yield and quality losses where growing-season rainfall was above-average and grain yields were >5 t/ha, which was similar to previous findings by Karamanos *et al*. (2012) in Canada. Fungicide application provided significant reductions in NFNB severity and improvements in grain yield and quality. Two applications of foliar fungicide provided the greatest improvements in grain yield (10–23%) and quality, with the treatment comprising foliar fungicide applications at Z31 + Z39 (T4) generally the most effective. This was comparable to previous findings of Martin and Sanderson (1988) in Canada and Stepanović *et al*. (2016) in Serbia. In our study, foliar fungicide applications at Z31 + Z39 or Z39 + Z55 were both effective. Of the foliar-fungicide application timings tested, application at Z39 was consistently effective in providing significant suppression. This was similar to the findings in a study by Turkington *et al*. (2012) in Canada, indicating that it is an effective application timing in different climatic zones and growing seasons and should be included in all foliar-fungicide management strategies for NFNB. Foliar fungicide application at Z31 provided significant grain yield improvements (7–10%) in two experiments but was less reliable than at Z39. There may

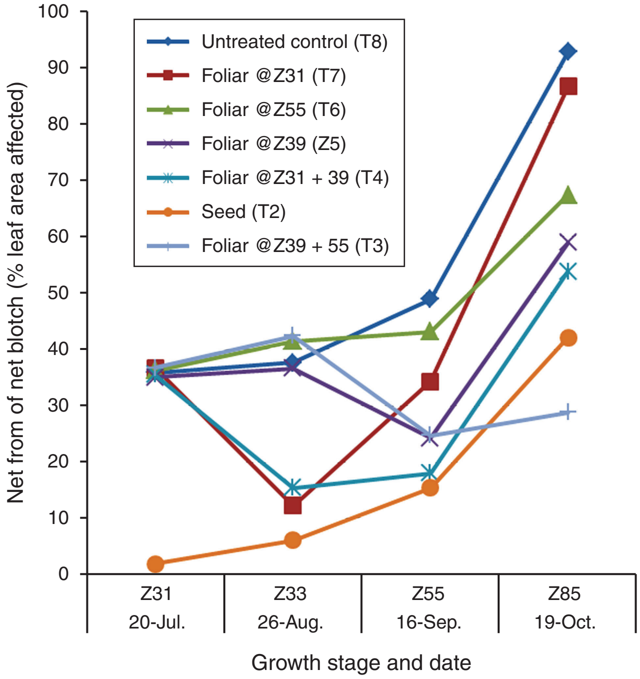

Fig. 1. Net form of net blotch severity of susceptible barley breeding line VB9613 at Z31–Z85 in response to seed-applied fluxapyroxad and foliar-applied prothioconazole + tebuconazole fungicide at different crop growth stages (Z31, stem elongation; Z39, flag leaf emergence; Z55, half ear emergence) at Vectis, Victoria, during 2016.

Table 3. Grain yield, percentage improvement in grain yield (gain) and grain quality relative to the untreated control of barley (breeding line VB9613) in response to foliar- and seed-applied fungicide treatments at Vectis during 2013 and 2016

Foliar treatments were Prosaro (a.i.s prothioconazole + tebuconazole at 210 + 210 g/L, applied at 300 mL/ha during 2012–14 and 150 mL/ha 2015–16). Seed treatment was Systiva (a.i. fluxapyroxad at 333 g/L, applied at 150 mL/100 kg seed). Growth stages: Z25, mid tillering; Z31, stem elongation; Z39, flag leaf emergence; Z55, mid ear emergence (Zadoks *et al*. 1974). –, Treatment not applied. *$P<0.05$; $^{\dagger}P<0.1$

Treatment	Grain yield				Grain quality				
	2013		2016		Retention (% >2.5 mm)		Screenings (% >2.2 mm)		1000-grain weight (g)
	(t/ha)	Increase	(t/ha)	Increase	2013	2016	2013	2016	2016
T1. Foliar Z25 + Z31 + Z55	5.2*	13%	–	–	79†	–	7†	–	–
T2. Seed	4.9*	8%	5.0*	20%	83*	51*	5*	15*	35*
T3. Foliar Z39 + Z55	5.0*	10%	5.2*	23%	85*	64*	4*	9*	37*
T4. Foliar Z31 + Z39	5.3*	15%	5.2*	23%	83*	47*	5*	14*	36*
T5. Foliar Z39	5.0*	10%	4.8*	17%	80*	50*	6*	16*	35*
T6. Foliar Z55	4.7†	4%	4.4*	9%	74	43†	10	20†	33
T7. Foliar Z31	5.0*	10%	4.3†	7%	74	35	9	24	33
T8. Untreated control	4.5	–	4.0	–	70	32	11	25	32
P-value	<0.001	–	<0.001	–	0.034	<0.001	0.021	<0.001	<0.001
l.s.d. (P = 0.05)	0.23	–	0.31	–	9.8	11.4	4.1	5.9	1.8
l.s.d. (P = 0.1)	0.19	–	0.25	–	8.1	9.5	2.4	4.9	1.5

Table 4. Correlations (*r*) of grain yield and quality to percentage leaf area affected by net form of net blotch on flag–flag-2 leaves in the barley breeding line VB9613 during 2013 and 2016

*$P<0.05$: significantly correlated; n.s. not significant

Leaf	Grain yield		Retention		Screenings		Grain weight	
	2013	2016	2013	2016	2013	2016	2013	2016
Flag	–0.508*	–0.734*	–0.624*	–0.753*	0.651*	0.712*	–0.495*	–0.681*
Flag-1	–0.632*	–0.843*	–0.713*	–0.816*	0.731*	0.800*	–0.503*	–0.747*
Flag-2	–0.629*	0.221n.s.	–0.676*	–0.101n.s.	0.676*	–0.150n.s.	–0.448*	–0.083n.s.

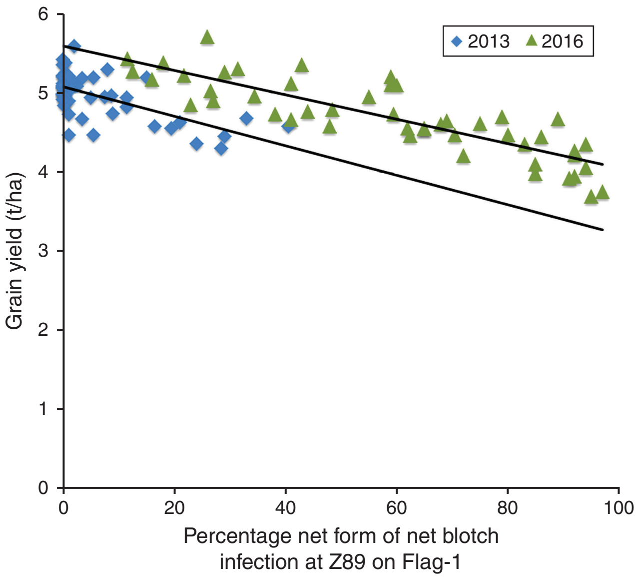

Fig. 2. Relationship between grain yield of barley breeding line VB9613 and average severity of net form of net blotch on the flag-1 leaf at grainfill (Z89) at Vectis during 2013 (yield = 5.08 – 0.0186 × disease severity; $P<0.05$) and 2016 (yield = 5.59 – 0.0154 × disease severity; $P<0.05$).

be merit in its inclusion in NFNB management strategies where disease pressure is high, although it would need to be followed up with a fungicide application at Z39. Foliar fungicide application at Z55 provided significant improvement in grain yield (4–9%) in two experiments ($P<0.05$ and $P<0.1$, respectively) and improvements ($P<0.1$) in grain quality during 2016. This finding was similar to those of Sutton and Steele (1983), Entz *et al*. (1990) and Turkington *et al*. (2004), who found that foliar fungicide application at ear emergence was generally useful in improving grain quality and yield. However, it was less beneficial in this study than in previous studies conducted in Canada. These findings support recommendations by Poole and Arnaudin (2014) that fungicide application needs to coincide with disease development, because foliar fungicide application provides short-term suppression of about 2–3 weeks (Sutton and Steele 1983). Our study showed that grain yield and quality losses were negatively correlated with NFNB infection on the flag to flag-1 leaves in one experiment and flag to flag-2 in another experiment. This demonstrated that fungicide strategies need to protect the top three leaves to minimise loss due to NFNB.

This study highlighted the difficulty of managing NFNB during seasons conducive to disease development, and that more than two applications of fungicide may be necessary to minimise loss in susceptible varieties. None of the fungicide strategies used in our study provided control of NFNB at Vectis during 2016. Fungicide application at Z31 did not provide much protection in relation to disease development post-application, especially after Z33 (Fig. 1), and significant disease development had already occurred before the single application at Z55. The treatment with three foliar fungicide applications tested at Vectis during 2013 provided good NFNB suppression but little benefit to grain quality. This was likely due to the inclusion of a less-effective application at Z25, compared an application at Z39, which provided better protection of the upper canopy leaf tissue and promoted grain-fill. Foliar fungicide application before stem elongation likely had little benefit for NFNB suppression and grain yield and quality. Sutton and Steele (1983) showed that foliar fungicide application at tillering could even increase NFNB development later in the season.

This is the first report on use of fluxapyroxad (Systiva) seed treatment for NFNB in barley. Our study showed significant reductions in NFNB severity and improvements in grain yield (8–20%) and quality compared with the untreated control. A previous study by McLean and Hollaway (2018) showed that fluxapyroxad was also effective in suppressing scald, indicating that it is a good option for barley disease management when combined with a follow-up application of foliar fungicide, especially where conditions are conducive to disease development post stem elongation.

This study demonstrates the importance of timely fungicide application for NFNB management and the role of the seed-applied fungicide fluxapyroxad. These strategies provide barley growers with flexibility when managing NFNB. This study also highlights the need to avoid relying solely on fungicides for NFNB control and the importance of using an integrated disease-management approach that incorporates crop rotation and growing host-plant resistant varieties to minimise risk of loss.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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