Effect of fungicide combination and timing on net form net blotch in barley – Grafton 2023

Sam Blanch[1], Natalie Moore[1], Ashley Moss[1] and Kefei Chen[2]

[1] NSW DPI, Grafton, NSW

[2] Curtain University, WA, Analysis for the Australian Grains Industry (AAGI)

Key findings

- Eight treatments incorporating fungicides from 3 mode of action (MOA) Groups (3, 4 and 7) were evaluated for efficacy and cost-effectiveness in managing net form net blotch (NFNB) in barley varieties with varying levels of genetic resistance: Fathom⏀ (susceptible), Zena CL⏀ (moderately susceptible) and Rosalind⏀ (moderately resistant).
- The treatments were combinations of genetic resistance, fungicide MOA group and application timing.
- The highest yielding treatment combination (6.68 t/ha) was variety Rosalind⏀ with Vibrance® seed treatment and Aviator® Xpro® applied at GS31. This was also the most cost-effective treatment combination, with a return on investment from fungicide applications at $2,269/ha, based on a grain price of $350/t.
- Seasonal conditions in 2023 were drier and warmer than average for this location and disease development later in the season was not as severe as expected in a year with wetter and cooler growing conditions.

Keywords: barley, net form net blotch, integrated disease management, IDM, *Pyrenophora teres*, fungicide

Introduction

The disease 'net form net blotch' (NFNB), caused by the fungus *Pyrenophora teres* f. *teres*, is estimated to be responsible for 18–31% yield loss annually in Australian barley crops. The pathogen survives on infected stubble and is also confirmed to spread in planting seed retained from infected crops.

Disease management decisions are complicated by factors including fungicide mode of action (MOA), timing of application, seasonal conditions, cost/ha, predicted yield and profit, and predicted disease pressure. Furthermore, resistance to some fungicide MOA Groups (3 and 7) has been confirmed in Australian NFNB populations. A key strategy to minimise the development of fungicide resistance is to rotate MOA groups and even active ingredients within groups. Hence, the need to evaluate various fungicide options in this study.

The research investment 'Integrated management strategies for NFNB in low, medium, and high rainfall zones' (GRDC code DAQ2304-008RTX) started in winter 2023 and aims to identify cost-effective integrated disease management (IDM) strategies for NFNB at field sites across eastern and southern Australia. The project is led by DAF QLD with multiple state and federal partners, including NSW DPI field sites in high (Grafton) and low (Wagga Wagga) rainfall zones.

The combined data from all sites will be analysed by Analysis for the Australian Grains Industry (AAGI) and reported separately by DAF QLD.

This report presents data from the first high rainfall field experiment for northern NSW conducted at NSW DPI Grafton in winter 2023. Experiments are planned for 3 consecutive seasons from 2023 to 2025.

Site details

Location	NSW DPI, Grafton Primary Industries Institute, Trenayr, Grafton, NSW. Latitude 29°31' 29.67" S, Longitude 150°45'12.64" E.
Paddock history	• 2022–23 summer Sunn hemp (*Crotalaria juncea*) • 2022 winter triticale (x *Triticosecale*) • 2021–22 summer teff grass (*Eragrostis tef*).
Soil type	• Red-brown clay • Table 1 details the soil chemical analysis for the experiment site.

Table 1 Soil chemical analysis Paddock 19A, NSW DPI Grafton (EAL Laboratory Services).

Characteristic/analyte	Unit	Soil depth		
		0–10 cm	10–30 cm	30–60 cm
pH (1:5 water)	pH units	6.4	6.0	6.0
Electrical conductivity (1:5 water)	dS/m	0.05	0.04	0.05
Aluminium (Al) Base saturation	%	0.3	1.4	0.6
Boron (Bo) 12C2 hot $CaCl_2$	mg/kg	0.4	0.4	0.5
Carbon (C) total	%	1.7	1.4	1.0
Carbon:nitrogen	ratio	10.2	10.2	9.5
Organic matter (OM) LECO Trumac	%	3.1	2.5	1.8
Chloride estimate	mg/kg	31.9	27.8	31.0
Copper (Cu) DTPA	mg/kg	1.0	0.8	0.4
Iron (Fe) DTPA	mg/kg	36.9	32.6	15.9
Magnesium (Mg) Morgan 1	%	18.8	18.0	19.6
Manganese (Mn) DTPA	mg/kg	92.4	81.9	32.1
Nitrogen (N) ammonium-N KCl extractable	mg/kg	3.7	3.3	2.9
Nitrogen (N) nitrate-N KCl extractable	mg/kg	7.4	6.4	6.5
Nitrogen (N) total LECO Trumac	%	0.2	0.1	0.1
Phosphorous (P) Bray 1	mg/kg	10.4	5.1	2.9
Phosphorus (P) Colwell	mg/kg	28.9	14.7	11.6
Silicon (Si) S11 hot $CaCl_2$	mg/kg	34.5	31.4	26.1
Sulfur (S) KCl_40	mg/kg	11.4	18.4	32.2
Zinc (Zn) DTPA	mg/kg	0.9	0.7	<0.5
Effective cation exchange capacity (ECEC)	cmol(+)/kg	6.9	5.8	5.9
Exchangeable aluminium (Al)	cmol(+)/kg	0.02	0.06	0.03
Exchangeable calcium (Ca)	cmol(+)/kg	4.9	4.3	4.4
Exchangeable magnesium (Mg)	cmol(+)/kg	1.3	1.0	1.1
Exchangeable potassium (K)	cmol(+)/kg	0.6	0.4	0.3
Exchangeable sodium (Na)	cmol(+)/kg	0.1	0.1	0.1
Calcium:magnesium	ratio	3.9	4.2	3.9

Temperature and rainfall	A total of 94.8 mm of rain fell during the growing season, which was 238.1 mm less than the long-term average (332.9 mm) for the same period (Figure 1). A total of 225 mm of supplementary overhead irrigation was applied throughout the latter dry part of the growing season to encourage disease development, making the total in-crop water received 319.8 mm.

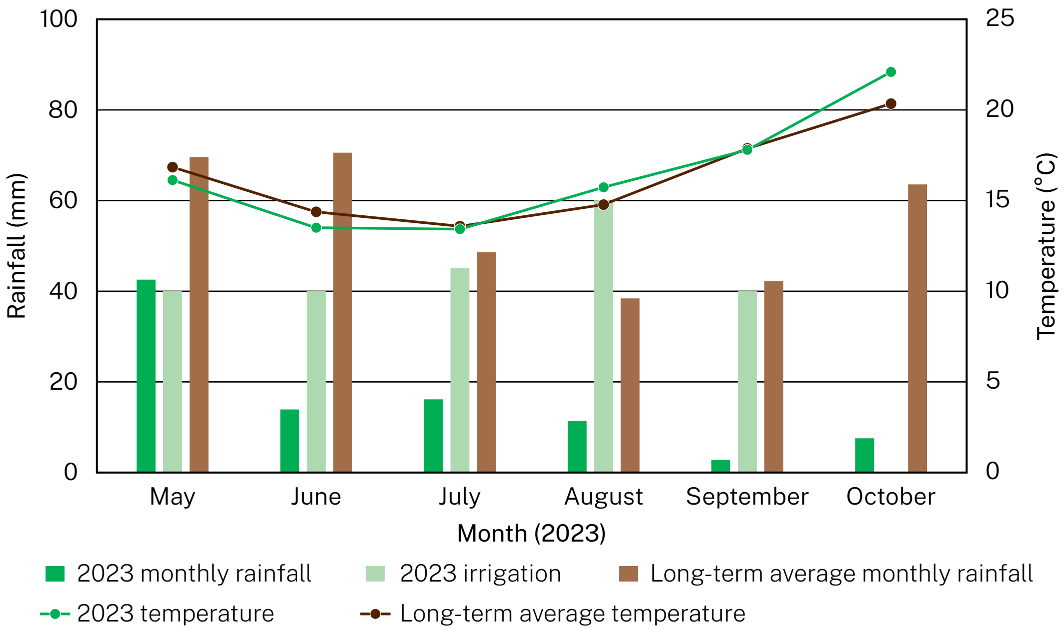

Figure 1 Temperature, rainfall and supplementary overhead irrigation for the 2023 growing season compared with long-term averages (LTA) at Grafton, NSW.

Sowing date	19 May 2023.
Fertiliser	• Sulfate of potash (41.5% K, 17% S) at 120 kg/ha broadcast 2 weeks before planting. • Diammonium phosphate (DAP) (17.7% N, 20% P) at 50 kg/ha broadcast at planting. • Triple super phosphate (45% P, 15% Ca) at 50 kg/ha banded with seed at planting. • Urea (46% N) at 70 kg/ha broadcast at GS20.
Plant population	• Target population: 150 plants/m^2. • Established population: 151 plants/m^2.
Weed management	Fallow applications: Crucial 600® at 2 L/ha (600 g/L glyphosate), Dicamba 500 at 200 mL/ha (500 g/L dicamba as dimethylamine salt) + Fusilade®Forte 820 mL/ha (128 g/L fluazifop-P). Post-emergence applications: Starane Advanced® 900 mL/ha (333 g/L fluroxypyr), Dicamba 500 at 200 mL/ha (500 g/L dicamba as dimethylamine salt).

Insect management	Pirimor® (500 g/kg pirimicarb) 300 g/ha to control corn aphid (*Rhopalosiphum maidis*).
Experimental design	The experiment was designed as a randomised split plot design with fungicide treatment as the main plot (8 levels) and variety as the sub-plot (3 levels) and 3 field replicates.
Harvest date	Physiological maturity (Zadok's growth stage GS93) was reached by all varieties on 6 October 2023, 140 days after sowing (DAS). Harvest was completed on 11 October 2023, 145 DAS.

Treatments

Varieties

Three varieties with a range of susceptibility to NFNB were included as experimental treatments:

1. Fathom⦶ – susceptible (S), local benchmark
2. Zena CL⦶ – moderately susceptible (MS)
3. Rosalind⦶ – moderately resistant (MR).

Net form net blotch management treatments

The fungicides used in this study are listed in Table 2 with the Group and mode of action (MOA). The treatment combinations applied and the product cost per hectare are detailed in Table 3. As application costs vary widely across farming systems, they have not been included. The growth stage (GS) for application was when each variety treated reached GS31 and/or GS39 according to the Zadok's scale.

Management for other barley diseases was not required this season.

Table 2 Fungicide groups and mode of action (MOA)included in NFNB experiment, NSW DPI Grafton 2023.

Fungicide active ingredient	Example trade name combining these active ingredients	Fungicide group	Fungicide MOA
Difenoconazole	Vibrance® seed treatment	3 (triazole)	De-methylation inhibitors (DMI), sterol biosynthesis (SBI)
Metalaxyl-M		4 (acylalanines)	Phenylamide, nucleic acid synthesis
Sedaxane		7 (pyrazole carboxamide)	Succinate dehydrogenase inhibitors (SDHI), respiration
Prothioconazole	Aviator® Xpro®	3 (triazole)	De-methylation inhibitors (DMI), sterol biosynthesis (SBI)
Bixafen		7 (pyrazole carboxamide)	Succinate dehydrogenase inhibitors (SDHI), respiration
Prothioconazole and tebuconazole	Prosaro®	3 (triazole)	De-methylation inhibitors (DMI), sterol biosynthesis (SBI)

Table 3 Fungicide treatments and cost per hectare for NFNB experiment, NSW DPI Grafton 2023.

Fungicide treatment			
Number	Code	Product and application timing[1]	Product cost ($/ha)[2]
1	Control (NIL)	Nil	0
2	VIB	Vibrance® seed treatment only	9
3	VIB+AVX@GS31	Vibrance® seed treatment + Aviator® Xpro® applied at GS31	69
4	VIB+PSR@GS31	Vibrance® seed treatment + Prosaro® 420 SC applied at GS31	36
5	AVX@GS31	Aviator® Xpro® applied at GS31	60
6	PSR@GS31	Prosaro® applied at GS31	27
7	VIB+PSR@GS31 +AVX@GS39	Vibrance® seed treatment + Prosaro® 420 SC applied at GS31 + Aviator® Xpro® applied at GS39	96
8	VIB+AVX@GS31+ PSR@GS39	Vibrance® seed treatment + Aviator® Xpro® applied at GS31 + Prosaro® 420 SC applied at GS39	96

[1] Vibrance – 66.2 g/L difenoconazole, 16.5 g/L metalaxyl-M, 13.8 g/L sedaxane
Aviator® Xpro® – 150 g/L prothioconazole, 75 g/L bixafen
Prosaro® 420 SC – 210 g/L prothioconazole, 210 g/L tebuconazole

[2] Cost of treatment ($/ha) was calculated based on the 2024 market price of fungicides applied at the recommended rate. As application costs vary for each farming system, these have not been included.

Results

Crop establishment

All varieties and plots established evenly across the site (Figure 2). Competition from weeds was low during the season.

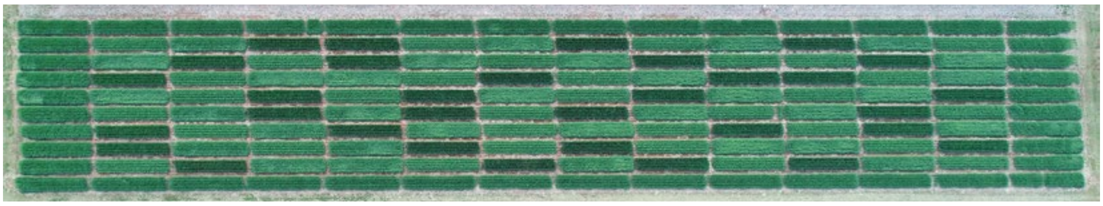

Figure 2 Aerial photograph of the barley experiment on 13 July, 55 DAP showing uniform establishment across the site. Note the darker green of the plots of variety Rosalind⊕. Photo: S Blanch, NSW DPI.

Disease development

Although the experiment was not sown into infected stubble this season, natural infection developed across the site. No additional inoculation was applied. Symptoms of NFNB were initially observed developing on the lower leaves of the MS variety Zena CL⊕ (Figure 3).

Conditions were conducive to natural infection and disease development early to mid-season, but the drier than average conditions later in the season slowed the disease development. Supplementary overhead irrigation was applied to encourage disease development. Overall, disease pressure at this site this season is best described as moderate. Table 3 presents disease severity scores.

Isolations were made from leaf lesions, of *Pyrenophora teres* f. *teres* pathotype was confirmed by Dr Lisle Snyman, DAF QLD, as the most similar to pathotype NB73, which is commonly recorded in barley crops across NSW. Leaf, stubble and grain samples from this experiment were provided to Dr Anke Martin, USQ, for molecular marker analysis to assess population structure over time (results reported separately).

The experiment matured evenly (Figure 4) and was harvested on 11 October, 145 DAS.

Figure 3 Net form net blotch leaf symptoms developing on moderately susceptible variety Zena CL$^{\phi}$, NSW DPI Grafton. Photo: N Moore, NSW DPI.

Figure 4 Agronomists inspecting an NFNB experiment at NSW DPI Grafton on 19 September, 123 DAS. Harvest was completed on 11 October, 145 DAS. Photo: N Moore, NSW DPI.

Statistical analysis

Linear mixed models were fitted with asreml-R (version 4.1.0) (Butler et al., 2018) in the analyses of the evaluated traits, where the variance parameters in the mixed models were estimated using the residual maximum likelihood (REML) procedure of Patterson and Thompson (Patterson and Thompson, 1971).

For each trait, spatial variations were examined, including local autocorrelations, global trends and extraneous variations. The blocking structures of the experiments were fitted as random effects. Spatial trends and residual variances with auto-regressive correlation at first-order for rows and columns were examined and fitted when the global trends and autocorrelations were significant. Likelihood ratio tests were used for random effects, and conditional Wald tests (Kenward and Roger, 1997) were used for fixed effects including variety, fungicide application and their interactions.

Residual diagnostics were performed to examine the validity of the model assumption of normality and homogeneity of variance. The empirical best unbiased linear estimations (eBLUEs) of fixed effects were produced from respective fitted models. The R package asremlPlus (Brien, 2020) was used to compute the least significant difference (LSD with α = 0.05) values for multiple comparisons.

Yield and grain quality

A summary of analysed data for disease severity score and grain yield is presented in Table 4, with a value for return on investment (ROI) for each fungicide treatment. Data in Table 4 are ranked from highest to lowest yield.

Table 4 Disease score, grain yield and ROI for fungicide treatments to manage NFNB of barley ranked from highest to lowest mean yield, NSW DPI Grafton 2023.

Variety treatment	Fungicide treatment number	Fungicide treatment code	Disease severity score[1] (1–9) at GS69	Yield (t/ha @ 11% moisture)	ROI (grain value less cost of fungicide) ($/ha)[2,3]
Rosalind	3	VIB+AVX@GS31	4.8	6.68[a]	2269
Rosalind	7	VIB+PSR@GS31+ AVX@GS39	3.0	6.52[a]	2186
Rosalind	5	AVX@GS31	4.8	6.43[a]	2192
Rosalind	4	VIB+PSR@GS31	5.5	6.30[a]	2168
Rosalind	8	VIB+AVX@GS31+ PSR@GS39	3.8	6.29[a,b]	2105
Rosalind	2	Vibrance seed treatment only	5.8	6.23[b]	2171
Rosalind	6	PSR@GS31	5.3	6.22[b]	2149
Rosalind	1	Control (nil)	5.8	6.18[b]	2162
Zena CL	7	VIB+PSR@GS31+ AVX@GS39	6.8	5.93[b]	1980
Zena CL	8	VIB+AVX@GS31+ PSR@GS39	6.5	5.92[b,c]	1977
Zena CL	6	PSR@GS31	7.8	5.79[c]	1999
Fathom	7	VIB+PSR@GS31+ AVX@GS39	5.3	5.77[c]	1925
Zena CL	3	VIB+AVX@GS31	8.0	5.77[c]	1952
Zena CL	5	AVX@GS31	8.0	5.72[c]	1941
Fathom	5	AVX@GS31	5.8	5.58[c]	1892
Fathom	3	VIB+AVX@GS31	5.5	5.56[c,d]	1878
Zena CL	1	Control (nil)	8.3	5.52[d]	1931
Fathom	6	PSR@GS31	6.8	5.45[d]	1882
Zena CL	2	Vibrance seed treatment only	8.5	5.45[d]	1899
Fathom	8	VIB+AVX@GS31+ PSR@GS39	5.3	5.43[d]	1804
Zena CL	4	VIB+PSR@GS31	8.0	5.27[d,e]	1808
Fathom	2	Vibrance seed treatment only	7.3	5.16[e]	1797
Fathom	1	Control (nil)	6.8	5.15[e]	1802
Fathom	4	VIB+PSR@GS31	6.0	5.12[e]	1756
		LSD (α = 0.05)	0.96	0.395	

[a-e] Treatment yields with the same letter are not statistically different from each other based on the least significant difference (LSD with α = 0.05) value of 0.395 t/ha.

[1] Disease severity score is based on the NVT scoring system of 1 (no symptoms present) to 9 (severe disease symptoms), taking account of the height of advancement of symptomatic leaves up the plant.

[2] Assumes grain price of $350/t delivered (barley grade 1, 2023 average price).

[3] Cost of treatment ($/ha) calculated on 2023 fungicide costs applied at the recommended rate. As application costs vary for each farming system, these have not been included.

The disease severity ratings for each of the varieties and treatments was analysed allowing comparison of treatments within and between varieties for disease development is presented in Figure 5. Note that in Figure 5, the varieties are ordered from left to right from the lowest disease severity rating (Rosalind⁽ᵖ⁾) to the highest disease rating (Zena CL⁽ᵖ⁾).

Figure 6 presents the analysed yield data for each of the varieties and treatments allowing comparison of treatments within and between varieties in terms of yield.

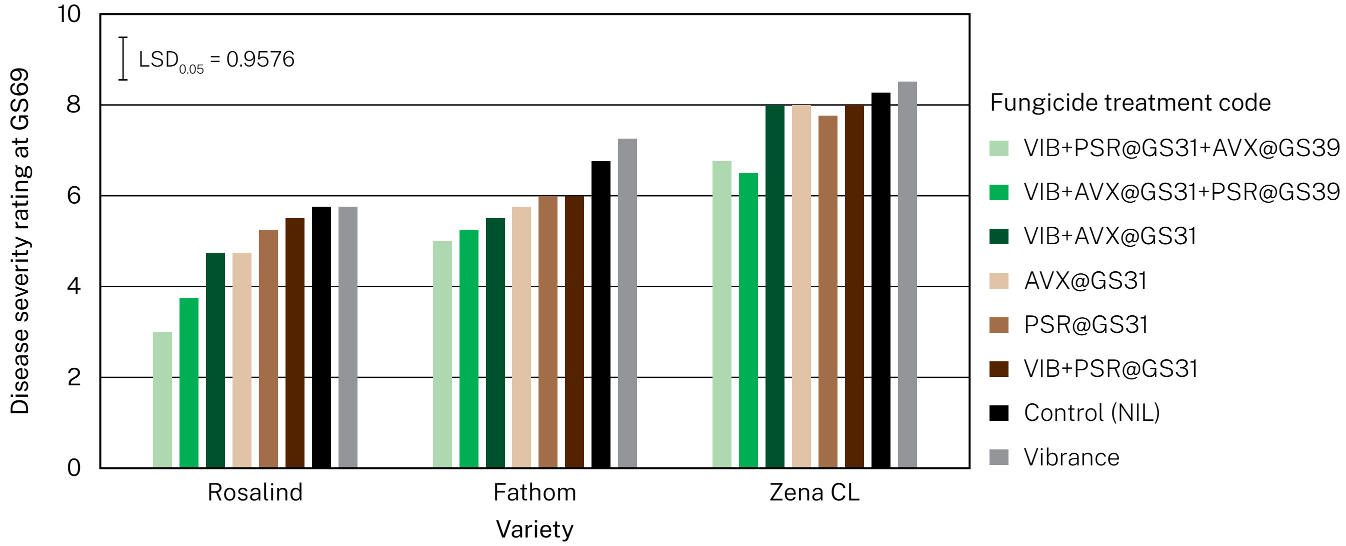

Figure 5 NFNB disease severity rating at GS69 for 3 barley varieties with different resistance ratings to NFNB (Rosalind⁽ᵖ⁾ MR, Zena CL⁽ᵖ⁾ MS and Fathom⁽ᵖ⁾ S) and with different fungicide treatments.

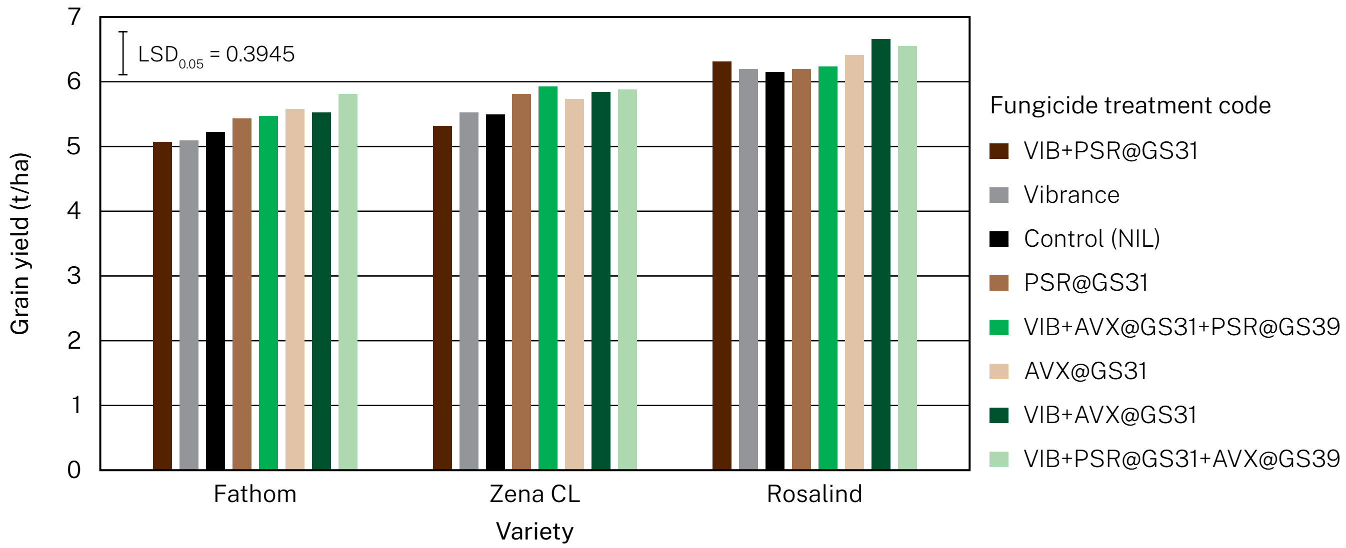

Figure 6 Grain yield for 3 barley varieties with different resistance ratings to NFNB (Fathom⁽ᵖ⁾ S, Zena CL⁽ᵖ⁾ MS and Rosalind⁽ᵖ⁾ MR) and with different fungicide treatments.

Economic analysis

The ROI of the treatments uses a price of $350/t (barley grade 1, 2023 average). The treatments are ranked from highest to lowest yield (Table 4). In a season with higher disease pressure, these rankings and the ROI values may change.

The ROI calculation was:

ROI = (grain price [$/t] × yield [t/ha]) − (fungicide treatment product cost [$/ha])

Grain quality results are presented in Table 5. Screenings from Zena CL⁽ᴰ⁾ treatments 1–5 are significantly higher than all other treatments. Most of the Fathom⁽ᴰ⁾ treatments had a significantly higher protein concentration than all other treatments.

Table 5 Grain quality results for NFNB of barley experiment, NSW DPI Grafton 2023.

Variety	Fungicide treatment number	Screenings (%)	1000 seed weight (g)	Test weight (kg/hL)	Grain protein concentration (% DMB)
Rosalind (MR)	1	3.97	45.19	67.23	13.23
	2	4.28	44.66	66.28	13.19
	3	3.20	46.36	66.93	13.74
	4	4.19	45.58	67.08	13.64
	5	3.56	45.94	67.76	13.56
	6	3.11	45.62	67.09	13.36
	7	3.21	46.53	67.85	13.12
	8	3.19	45.92	67.66	13.26
Zena CL (MS)	1	8.84	41.91	61.31	13.69
	2	6.34	41.04	60.29	13.39
	3	5.46	42.54	61.71	13.27
	4	6.94	40.93	60.16	13.70
	5	6.09	43.44	61.17	13.43
	6	3.99	42.77	62.22	13.32
	7	3.51	44.51	63.88	13.17
	8	3.94	43.91	63.24	13.27
Fathom (S)	1	3.31	49.72	61.78	14.20
	2	2.71	49.32	64.41	14.35
	3	2.63	49.17	63.45	14.11
	4	3.54	48.04	63.30	14.57
	5	2.71	51.07	61.74	14.56
	6	2.44	49.47	63.34	14.30
	7	1.76	50.78	64.64	14.30
	8	2.00	50.20	63.54	14.39
	LSD	1.625	2.449	2.673	0.549

LSD = least significant difference (with α = 0.05) value.

DMB = dry matter basis.

Conclusions

The 2023 season at Grafton had conditions conducive for natural (i.e. not inoculated) infection by the NFNB pathogen and moderate disease pressure early- to mid-season. However, during the remainder of the season, disease pressure did not continue to increase due to prolonged dry conditions experienced until harvest.

The highest yield was from the MR variety Rosalind⁽ᴰ⁾, which has the highest level of resistance to NFNB of the 3 varieties examined. Four of the fungicide treatments for Rosalind⁽ᴰ⁾ resulted in statistically similar yields (6.68 to 6.30 t/ha), highlighting the importance of choosing a variety with higher levels of genetic resistance to manage NFNB.

The most cost-effective disease control treatment under this level of disease pressure was variety Rosalind⁽ᴰ⁾ with Vibrance® seed treatment and Aviator® Xpro® applied at GS31, with a return on investment from fungicide applications at $2,269/ha. In seasons with earlier or more severe disease development (i.e. milder, wetter winters), the ROI values and rankings may vary.

If growers continue to use known NFNB-susceptible varieties such as Fathom⁽ᵇ⁾, they will likely increase the level of inoculum in the crop stubble, which then increases disease pressure in the following season. Using susceptible varieties reduces the ROI from fungicide treatments, even in a season with moderate disease pressure, such as 2023.

In this experiment, growing an MR-rated cultivar such as Rosalind⁽ᵇ⁾ increased the yield and ROI from fungicide. It also reduced the overall inoculum load left behind in the stubble, which reduced the inoculum load at the start of the following season.

The Vibrance® seed treatment alone performed statistically similar to the control for each cultivar, suggesting the seed used for this experiment had little to no infection from NFNB.

The NVT disease resistance rating to NFNB for variety Zena CL⁽ᵇ⁾ is MS and Fathom⁽ᵇ⁾ is S (online NVT ratings January 2023). However, in this experiment Zena CL⁽ᵇ⁾ developed symptoms early in the season and had disease rating scores higher than Fathom⁽ᵇ⁾ for most treatments. The NFNB pathotype in this experiment was confirmed as being most similar to pathotype NB73, which is widespread in NSW.

Zena CL⁽ᵇ⁾ also produced higher grain screenings and a lower 1,000 seed weight overall compared with Rosalind⁽ᵇ⁾ and Fathom⁽ᵇ⁾, which could be attributed to its higher disease development ratings at GS69.

Variety and fungicide choice are particularly important in seasons with high yield potential and when milder (15–25 °C) and wetter than average winter conditions are predicted (e.g. La Niña cycles), and when crops are planted into NFNB infected stubble from previous barley crops.

In this experiment the Prosaro® (210 g/L prothioconazole, 210 g/L tebuconazole) treatments did not suppress disease development as well as the Aviator® Xpro® (150 g/L prothioconazole, bixafen 75 g/L) treatments. In some cases, the Prosaro® treatment was statistically the same as the nil control treatment. This result could suggest this strain of NFNB has developed resistance to this fungicide in this region. Resistance testing is required to confirm this.

Resistance of the NFNB pathogen to fungicide Group 3 (DMI) and Group 7 (SDHI) has been reported in several states in Australia. This is an important consideration when evaluating the efficacy of fungicide treatments and selecting disease management options to minimise the development of further resistance in the pathogen. The GRDC fact sheet ‘Fungicide resistance in barley crops: causes, management and mitigation strategies’ October 2021 provides more information.

Growing back-to-back barley crops is not a recommended practice. However, when this is unavoidable and a crop is sown into NFNB-infected stubble, growers are encouraged to consider using a variety with resistance to NFNB and to invest in the timely application of fungicide to reduce the risk of losses to this disease.

References

Brien C (2020) AsremlPlus: Augments ‘ASReml-R’ in fitting mixed models and packages generally in exploring prediction differences. R package version 4.3.55. http://chris.brien.name/rpackages

Butler DG, Cullis BR, Gilmour AR, Gogel BJ and Thompson R (2018) ASReml-R reference manual version 4, VSN International Ltd, Hemel Hempstead, HPI 1ES, UK.

Kenward MG and Roger JH (1997) Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, 983-997.

Patterson HD and Thompson R (1971) Recovery of inter-block information when block sizes are unequal. *Biometrika* 58, 545–554.

Acknowledgements Dr Ben Ovenden and Brad Baxter, NSW DPI Wagga Wagga, are gratefully acknowledged for technical advice. Dr Steven Simpfendorfer, NSW DPI Tamworth, is gratefully acknowledged for technical advice and review of the report. Dr Lisle Snyman and Dr Xuechen Chen, DAF QLD, are gratefully acknowledged for isolate pathotyping. Nathan Ensbey and Nathan Collins, NSW DPI Grafton, are gratefully acknowledged for technical assistance in planting and harvesting the experiment.

This work is a component of 'Program 5: Integrated management strategies for Net Form Net Blotch of barley in low, medium, and high rainfall zones' a co-investment by GRDC, DAFQ, NSW DPI, SARDI, and FAR Australia (GRDC Project code DAQ2304-008RTX).

Published by NSW Department of Primary Industries, a part of the Department of Regional New South Wales.