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Effects of fungicide chemical class, fungicide application timing, and environment on Fusarium head blight in winter wheat

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Abstract Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, can result in devastating economic losses in small grain cereal crops. Management of FHB is by a combination of strategies and tactics including cultivar resistance, fungicide application at anthesis, and cultural practices such as crop rotation, tillage, and irrigation management. This study evaluated, under field conditions, the effects of fungicide chemical class (triazole versus strobilurin), fungicide application timing, and environment on FHB and its associated mycotoxin deoxynivalenol (DON). A moderately resistant hard red winter wheat cultivar, Overland, consistently had lower levels of FHB index (= index), *Fusarium*-damaged kernels (FDK), and DON, and higher yield compared to the susceptible hard red winter wheat cultivar Overley. The most effective fungicide treatment in reducing FHB, FDK, DON, and yield loss was Prosaro® (prothioconazole + tebuconazole) applied at early anthesis (BBCH 61; hereafter referred to as anthesis). Application of Prosaro 6 days post anthesis (DPA) achieved a slightly lower but comparable efficacy to that achieved by the anthesis application. Application of Prosaro at 12 DPA was least effective. The strobilurin fungicide Headline® (pyraclostrobin) was less effective than Prosaro in controlling FHB, FDK, and DON. In both cultivars, index, FDK, and DON were higher and yield was lower under irrigated compared to rain-fed conditions. These differences were more pronounced in a wet compared to a relatively dry growing season. The results from this study indicate that effective management of FHB can be achieved by combining cultivar resistance with a triazole fungicide applied at anthesis, and the window of fungicide application can be extended by up to 6 days post anthesis.

Keywords *Fusarium graminearum* · Triazole · Strobilurin · Index · Deoxynivalenol (DON) · *Fusarium*-damaged kernels (FDK) · Thousand kernel weight (TKW) · Yield

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Introduction

Fusarium head blight (FHB) or scab is a devastating disease of wheat and other small grain cereal crops. In the United States, the main causal agent of FHB is

Fusarium graminearum Schwabe (Dill-Macky 2010), but there are other causal agents grouped within the *Fusarium graminearum* species complex (Aoki et al. 2012). FHB causes economic losses not only in yield and reduced grain volume weight, but due to accumulation of the mycotoxin deoxynivalenol (DON, vomitoxin) in grain. FHB pathogens survive intercrop periods as mycelia, chlamydospores, or perithecium initials in host crop residues (Khonga and Sutton 1988). Primary inoculum consists of ascospores discharged from mature perithecia in the spring. The ascospores are transported in air currents, land on spikes, and infect the spikelets mostly during anthesis (Zadoks growth stages BBCH 60–69; Zadoks et al. 1974). A typical symptom of FHB is premature bleaching or whitening of the spike. Infected spikelets are sterile or contain shriveled, chalky white or pink kernels known as *Fusarium*-damaged kernels (FDK) or 'tombstones' which are unsuitable for processing into food products (McMullen et al. 1997).

FHB is favored by abundant rainfall before, during, and following anthesis; high relative humidity (RH); moderate to warm temperatures; and reduced tillage practices that leave cereal crop residue on the soil surface (Shaner 2003). In Nebraska, recent major outbreaks occurred in 2007, 2008, 2015, and 2019 (Lilleboe 2015, 2019; McMullen et al. 2012; Mengistu et al. 2007; Wegulo et al. 2008). Although considerable research efforts have been undertaken to develop effective management strategies and tactics for FHB, the disease continues to be a major challenge for growers. The acreage of wheat and barley have declined drastically due to, among other factors, FHB epidemics (McMullen et al. 2012).

Fungicide application is one of the management tactics for FHB. Fungicide chemical class and optimal application timing are critical for effective management of the disease. Efficacy of triazole-based fungicides in controlling FHB and DON has been demonstrated in field trials (Paul et al. 2008). Fungicide application is recommended at anthesis because the most damaging infections occur on wheat spikes during that growth stage or thereafter (Andersen 1948). The narrow window (about 2 days) of fungicide application at early anthesis presents challenges to the grower who may be unable to apply at that time due to various reasons including unfavorable weather (wind, rainfall), scheduling with commercial applicators, or unavailability due to personal commitments. A wider application window can provide needed flexibility to the grower.

Strobilurin fungicides are effective in controlling foliar fungal diseases of wheat (Wegulo et al. 2009, 2011b); however, they have been associated with elevated levels of DON when applied at or before anthesis (Amarasinghe et al. 2013; Ellner 2005; Madden et al. 2014; Pirgozliev et al. 2002; Simpson et al. 2001), although this observation has been inconsistent, thereby necessitating further research.

Genetic resistance is one of the most effective strategies for managing plant diseases including FHB. In 2006, the moderately resistant winter wheat cultivar Overland with adaptation to rain-fed conditions of Nebraska, South Dakota, and the northern Great Plains was released by the Nebraska wheat breeding program (Baenziger et al. 2008). The susceptible winter wheat cultivar Overley was released in Kansas in 2003 (Fritz et al. 2004) for its high yields. Knowledge of the reaction of moderately resistant and susceptible wheat cultivars to triazole and strobilurin fungicides will be useful in developing more effective management strategies for FHB. The objectives of this study were to evaluate, under Nebraska field conditions, the effects of: 1) fungicide chemical class, 2) fungicide application timing, and 3) environment on FHB index (hereafter referred to as index), *Fusarium*-damaged kernels (FDK), DON, thousand kernel weight (TKW), and yield in a moderately resistant and a susceptible winter wheat cultivar. Correlations were conducted to determine the relationships among index, FDK, DON, TKW, and yield.

Materials and methods

Field experiments were conducted under rain-fed (one experiment) and irrigated (one experiment) conditions during each of the 2015 and 2016 wheat growing seasons at the Eastern Nebraska Research and Extension Center (ENREC) near Mead, Nebraska (41.2286° N, 96.4892° W). Two hard red winter wheat cultivars, Overland (moderately resistant to FHB; hereafter referred to as the MR cultivar), and Overley (susceptible to FHB; hereafter referred to as the S cultivar) were seeded in clean tilled soil in the autumn of 2014 and 2015 at a rate of 101 kg ha^{-1}. Soil was fertilized with 78 kg N ha^{-1} before seeding. Standard practices for weed control with herbicides were followed. The previous crop at the sites where the rain-fed experiment was established in both years was maize. The sites where the irrigated experiment was established in both years were fallow before clean tilling and seeding in the autumn. Plots measured 1.22 m × 6.10 m in 2015 and 1.22 m × 4.57 m in 2016.

Daily weather data for the research location (Mead, Nebraska, USA) were provided by the High Plains Regional Climate Center's Automated Weather Data Network (High Plains Regional Climate Center, School of Natural Resources, University of Nebraska-Lincoln, Lincoln, Nebraska, USA). Average normal precipitation data for the research location were obtained from the United States National Centers for Environmental Information (NCEI), Asheville, North Carolina. In the irrigated experiments, impact sprinklers (model 30H, Rain Bird, Azusa, CA) were set to irrigate the plots for 5 min at 15-min intervals from 10.00 to 17.00 each day. Irrigation started just before anthesis in late May and continued for approximately 3 weeks into June. Precipitation in the irrigated experiments was measured with Watchdog® portable weather stations (Spectrum Technologies, Thayer Court, IL) placed in plot alleys. During the irrigation period, precipitation (rainfall and irrigation) averaged 24 mm/day in 2015 and 23 mm/day in 2016.

The experimental design was a split plot in randomized complete blocks with four replications, with cultivars as the main plots. Each fungicide treatment (including the check) and its timing was a subplot. There were two cultivars (Overland (MR) and Overley (S)) and seven fungicide treatments: 1) Non-treated check, 2) Prosaro (tebuconazole + prothioconazole) applied at early anthesis (BBCH 61; hereafter referred to as anthesis), 3) Prosaro applied 6 days post anthesis (DPA), 4) Prosaro applied 12 DPA, 5) Headline (pyraclostrobin) applied at anthesis, 6) Headline applied 6 DPA, and 7) Headline applied at 12 DPA. Prosaro was applied at a rate of 0.090 L ha^{-1} prothioconazole +0.090 L ha^{-1} tebuconazole. Headline was applied at a rate of 0.155 L ha^{-1} pyraclostrobin.

Plots were inoculated by spreading *F. graminearum*-colonized maize kernels at a rate of approximately 68 kernels m^{-2} weekly for 3 weeks beginning in late April. At early anthesis (BBCH 61) and 6 and 12 DPA, fungicides were applied to the spikes using a CO_2-powered backpack sprayer set at 241 kPa and equipped with four Tee-jet 800-LVS nozzles (Tee-Jet Technologies, Dillsburg, PA) angled forward about 30° and spaced 30.5 cm apart. A volume of 150 L ha^{-1} of spray mixture was delivered to the plots. Nonionic surfactant (NIS 90–10, Precision Laboratories, Inc., Waukegan, IL) was added to the spray mixture at 0.125% *v*/v. At 24 h after fungicide treatments, plots were inoculated by spraying the spikes with 27 mL m^{-2} of a suspension of *F. graminearum* spores using a hand-pumped SOLO® 485 backpack sprayer (*SOLO*® Inc., Newport News, VA). Spores were harvested from potato dextrose agar (PDA) culture plates on which five Nebraska *F. graminearum* isolates were grown for three to five weeks at room temperature. The spores from the different isolates were mixed in sterile distilled water and the concentration was adjusted to 100,000 spores mL^{-1}.

Index (percentage of symptomatic spikelets on all spikes sampled), was assessed on 100 spikes in each plot (20 spikes in each of five randomly selected spots in each plot) at 16 and 21 days after fungicide application in 2015 and 2016, respectively. At maturity, plots were harvested with a small plot combine (Wintersteiger, Dimmelstrasse, Austria) which recorded yield. The fan speed on the combine was set to minimize loss of FDK. The percentage of FDK was visually determined in subsamples of the harvested grain. Gas chromatography with electron capture detection (Tacke and Casper 1996) was used to measure DON at the North Dakota Veterinary Diagnostic Laboratory in subsamples of grain that were previously ground to flour.

Data analysis

Data were analyzed with SAS software version 9.4 (SAS Inc., Cary, NC) using linear mixed models. Due to heterogeneous error variances across experiments determined by the F-ratio test (Gomez and Gomez 1984), data from each experiment were analyzed separately. Fisher's least significant difference (LSD) test at $P = 0.05$ was used to compare pairs of treatment means. In this paper, when comparing treatment means, use of "did not differ", "higher", "greater", or "lower" is in reference to the LSD at $P = 0.05$. F-values for treatment effects were considered significant at $P \leq 0.05$. To determine the effect of irrigated or non-irrigated (rainfed) environment on FHB, FDK, DON, and yield, year was considered a replication and means of these variables for each cultivar were averaged over fungicide treatments. Treatment means were used in analysis of correlation (Gomez and Gomez 1984) between measured variables (index, FDK, DON, TKW, and yield) using PROC CORR in SAS.

Results

Weather conditions and cultivar responses

The critical period for FHB development is from anthesis to about three weeks later. Anthesis started during the last week of May. In 2015 during the 18 days following anthesis (28 May to 15 June), favorable natural conditions (rainfall, moderate to warm temperatures, and high RH) prevailed (Fig. 1). During this period, total rainfall was 112 mm whereas in the irrigated experiment total precipitation from rainfall and irrigation was 552 mm. Average daytime high temperature was 26 °C and average RH was 76%.

During the same period in 2016 (28 May to 15 June), natural conditions were unfavorable to FHB development with only 17 mm of rainfall during the 18 days following anthesis, accompanied with hot and dry conditions (Fig. 1). Average daytime high temperature was 31 °C and average RH was 58%. Total precipitation in

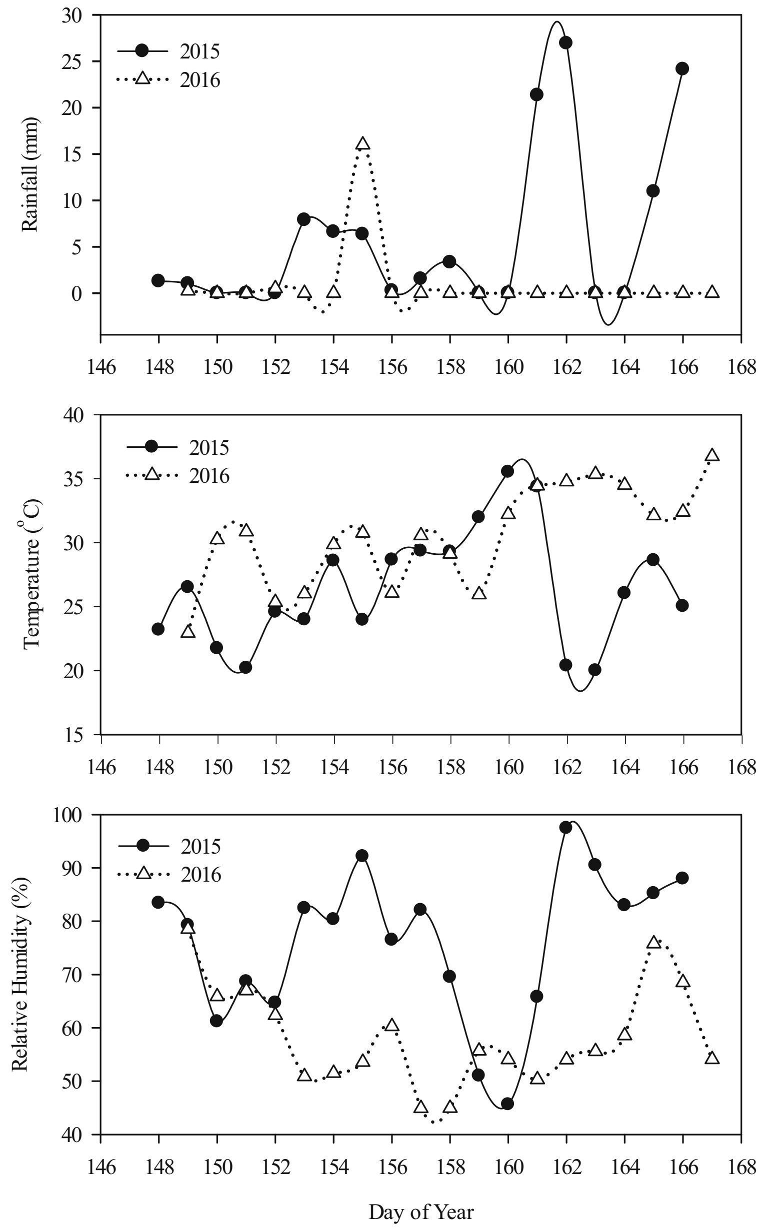

Fig. 1 Daily rainfall, high daytime temperature, and relative humidity from the Mead meteorological station near the experiment sites from May 28 to June 15 in 2015 (Day of Year 148–166) and 2016 (Day of Year 149–167). During this period, total rainfall was 112 mm in 2015 and 17 mm in 2016. Average rainfall at this location is 108 mm in May and 116 mm in June (United States National Centers for Environmental Information (NCEI), Asheville, North Carolina)

the irrigated experiment during the 18 days was 431 mm of which 414 mm was from irrigation.

The MR cultivar consistently had lower FHB index, FDK, and DON and yielded higher than the S cultivar (Tables 1 and 2). The exceptions were DON in the rain-fed experiment in 2016 and yield in the irrigated experiment in 2016. In these two experiments, the two variables were similar in the majority of treatments in both cultivars.

Rain-fed experiment, 2015

In the MR cultivar, both Prosaro (triazole) and Headline (strobilurin) resulted in lower index, FDK, and DON and higher TKW and yield than the check when applied at anthesis. Index was generally low and ranged from 6.2% in the Headline anthesis treatment to 13.5% in the check. FDK ranged from 11.5% in the anthesis treatments of both fungicides to 29.2% in the check. Prosaro timing treatments (anthesis, 6 DPA, 12 DPA) did not differ in FDK; however, for Headline, the anthesis treatment resulted in lower FDK than the 6 DPA and 12 DPA treatments. DON ranged from 6.2 μg g^{-1} in the Prosaro anthesis treatment to 16.3 μg g^{-1} in the check. The Headline anthesis treatment resulted in higher DON but also yielded higher than the Prosaro anthesis treatment (Table 1). For Prosaro, the anthesis treatment resulted in lower DON than both the 6 DPA and 12 DPA treatments. TKW ranged from 23.8 g in the check to 28.2 g in the Prosaro anthesis treatment. For both fungicides, the anthesis treatment resulted in greater TKW than the 6 DPA and 12 DPA treatments. Yield ranged from 1942 kg ha^{-1} in the check to 3087 kg ha^{-1} in the Headline anthesis treatment. Prosaro timing treatments did not differ in yield; however, for Headline, the anthesis treatment resulted in higher yield than the 6 DPA and 12 DPA treatments.

In the S cultivar, index ranged from 26.9% in the Prosaro 6 DPA treatment to 59.5% in the check and did not differ among treatments. FDK ranged from 43.2% in

Table 1 Means of Fusarium Head blight index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), thousand kernel weight (TKW), and yield from rain-fed and irrigated experiments conducted to determine the effects of fungicide chemical class, fungicide application timing, and environment on the variables in a moderately resistant (MR, Overland) and a susceptible (S, Overley) winter wheat cultivar during the 2015 growing season

Fungicide Treatment	Index (%)		FDK (%)		DON (μg/g)		TKW (g)		Yield (kg/ha)	
	MR	S	MR	S	MR	S	MR	S	MR	S
Rain-fed										
Check	13.5 a[a]	59.5 a	29.2 a	77.5 a	16.3 a	63.8 a	23.8 d	16.5 b	1942 c-e	677 c
Prosaro A	6.5 c	37.7ab	11.5 c	43.2 d	6.2 d	34.9 c	28.2 a	23.9 a	2531 b	1484 a
Prosaro 6 DPA	11.8 ab	26.9 b	16.5 bc	43.8 d	10.5 c	32.9 c	27.3 b	22.1 a	2159 b-d	1284 ab
Prosaro 12 DPA	7.4 bc	48.2 ab	17.8 bc	61.0 bc	11.3 bc	40.2 bc	25.0 c	18.4 b	2340 bc	1114 a-c
Headline A	6.2 c	43.8 ab	11.5c	48.0 cd	10.7 c	37.8 bc	28.4 a	21.4 a	3087 a	1468 a
Headline 6 DPA	10.5 a-c	39.6 ab	20.2 b	53.0 cd	9.6 c	37.8 bc	25.4 c	22.2 a	1874 de	1427 a
Headline 12 DPA	8.6 bc	50.2 ab	23.0 ab	74.0 ab	14.0 ab	45.6 b	24.9 c	18.0 b	1694 e	932 bc
P > F	0.0229	0.1838	0.0002	<.0001	0.0001	<.0001	<.0001	<.0001	0.0001	0.0122
Irrigated										
Check	38.6 a	80.3 ab	54.8 ab	79.2 a	46.1 a	78.2 a	21.5 b	19.8 c	1819 b	1122 bc
Prosaro A	19.2 a	40.2 d	22.0 e	41.5 e	7.8 d	19.1 c	24.6 ab	23.7 ab	2913 a	1656 a
Prosaro 6 DPA	22.7 a	56.1 cd	34.2 d	50.2 de	11.2 d	16.8 c	22.9 ab	25.1 a	2050 b	1272 a-c
Prosaro 12 DPA	28.4 a	72.7 a-c	45.8 bc	68.8 a-c	18.3 cd	40.2 b	24.5 ab	20.6 c	1480 b	988 c
Headline A	35.4 a	62.0 bc	42.8 cd	60.5 cd	37.2 ab	41.4 b	26.5 a	24.4 ab	2078 b	1525 ab
Headline 6 DPA	25.1 a	72.3 a-c	58.2 a	68.2 bc	18.2 cd	37.4 b	24.8 ab	22.2 bc	1864 b	1324 a-c
Headline 12 DPA	26.9 a	86.4 a	58.0 a	75.0 ab	29.1 bc	48.0 b	23.0 ab	19.6 c	1490 b	1146 bc
P > F	0.5029	0.0014	<.0001	<.0001	<.0001	<.0001	0.1909	0.0006	0.0293	0.0599

[a] Means followed by the same letter within a column in each environment (Rain-fed and Irrigated) are not significantly different according the least significant difference (LSD) test at P = 0.05

Table 2 Means of Fusarium Head blight index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), thousand kernel weight (TKW), and yield from rain-fed and irrigated experiments conducted to determine the effects of fungicide chemical class, fungicide application timing, and environment on the variables in a moderately resistant (MR, Overland) and a susceptible (S, Overley) winter wheat cultivar during the 2016 growing season

Fungicide Treatment	Index (%)		FDK (%)		DON (μg/g)		TKW (g)		Yield (kg/ha)	
	MR	S	MR	S	MR	S	MR	S	MR	S
Rain-fed										
Check	16.7 a[a]	42.4 a	7.2 a	11.0 a	0.1 a	1.2 ab	22.9 a	26.5 d	3113 cd	2730 cd
Prosaro A	4.5 d	19.6 d	5.7 ab	6.2 b	0.1 a	0.8 a-c	23.8 a	31.2 bc	3957 a	3542 ab
Prosaro 6 DPA	8.4 b-d	25.3 cd	7.8 a	6.0 b	0.1 a	0.4 c	23.3 a	34.8 a	3590 a-d	3159 bc
Prosaro 12 DPA	12.3 b	25.8 cd	4.2 b	7.2 ab	0.1 a	1.4 a	24.2 a	30.3 c	3077 d	2574 cd
Headline A	6.8 cd	29.9 bc	4.2 b	8.8 ab	0.1 a	1.0 a-c	24.6 a	33.2 ab	3744 ab	4061 a
Headline 6 DPA	11.4 b	34.9 ab	8.2 a	7.8 ab	0.2 a	0.6 bc	24.3 a	31.4 bc	3635 a-c	2756 b-d
Headline 12 DPA	9.9 bc	39.7 a	5.2 ab	10.2 ab	0.1 a	1.4 a	23.2 a	31.8 bc	3312 b-d	2207 d
P > F	0.0002	<.0001	0.0986	0.2235	0.4512	0.0659	0.4424	<.0001	0.0175	0.0023
Irrigated										
Check	38.6 a	73.5 a	25.5 ab	39.0 a	1.1 a	4.0 ab	21.2 ab	25.8 bc	2332 ab	1865 b
Prosaro A	16.3 b	34.2 de	13.8 c	21.5 b	0.6 b	2.9 ab	20.1 a-c	27.9 ab	2817 ab	3492 a
Prosaro 6 DPA	22.3 b	40.9 cd	25.2 ab	22.2 b	1.1 a	2.6 b	17.6 d	27.5 ab	2661 ab	2604 ab
Prosaro 12 DPA	32.6 a	54.5 b	24.2 ab	25.0 b	0.8 ab	3.7 ab	18.7 b-d	28.1 a	2165 b	2927 ab
Headline A	22.8 b	26.8 e	15.2 c	26.8 b	0.6 b	4.2 a	21.9 a	29.6 a	2945 a	3044 ab
Headline 6 DPA	34.1 a	46.8 bc	18.5 bc	26.2 b	0.8 ab	3.1 ab	19.1 b-d	27.6ab	2822 ab	2798 ab
Headline 12 DPA	38.1 a	67.2 a	27.0 a	43.2 a	1.2 a	4.1 ab	18.1 cd	25.1 c	2078 b	2376 ab
P > F	<.0001	<.0001	0.0060	0.0014	0.0706	0.2012	0.0138	0.0093	0.1313	0.2615

[a] Means followed by the same letter within a column in each environment (Rain-fed and Irrigated) are not significantly different according the least significant

the Prosaro anthesis treatment to 77.5% in the check. For both fungicides, the anthesis and 6 DPA treatments resulted in lower FDK than the check and 12 DPA treatments. DON ranged from 32.9 μg g^{-1} in the 6 DPA Prosaro treatment to 63.8 μg g^{-1} in the check. All fungicide treatments resulted in lower DON than the check. TKW ranged from 16.5 g in the check to 23.9 g in the Prosaro anthesis treatment. TKW from the anthesis and 6DPA treatments was higher than that from the 12 DPA treatments for both fungicides. Yield ranged from 677 kg ha^{-1} in the check to 1484 kg ha-1 in the Prosaro anthesis treatment. For Headline, the anthesis and 6 DPA treatments resulted in higher yield than the 12 DPA treatment whereas yield was similar among all Prosaro treatments (Table 1).

Irrigated experiment, 2015

In the MR cultivar, index ranged from 19.2% in the Prosaro anthesis treatment to 38.6% in the check and did not differ among treatments. FDK ranged from 22.0% in the Prosaro anthesis treatment to 58.2% in the Headline 6 DPA treatment. All Prosaro treatments and the Headline anthesis treatment resulted in lower FDK than the check and Headline 6DPA and 12 DPA treatments. For Prosaro, FDK from the anthesis treatment was lower than that from the 6 DPA treatment which was lower than that from the 12 DPA treatment. For Headline, FDK from the anthesis treatment was lower than that from both the 6 DPA and 12 DPA treatments. DON ranged from 7.8 μg g^{-1} in the Prosaro anthesis treatment to 46.1 μg g^{-1} in the check. DON from the Headline anthesis treatment did not differ from that in the check and was nearly five times the DON in the Prosaro anthesis treatment. TKW ranged from 21.5 g in the check to 26.5 g in the Headline anthesis treatment and did not differ among fungicide treatments. Yield ranged from 1819 kg ha^{-1} in the check to 2913 kg ha^{-1} in the Prosaro anthesis treatment. Yield in this treatment was higher than that in all other treatments which did not differ from each other (Table 1).

In the S cultivar, index ranged from 40.2% in the Prosaro anthesis treatment to 86.4% in the Headline 12 DPA treatment. Index in the Headline anthesis treatment was 21.8% higher than that in the corresponding Prosaro treatment. The Prosaro anthesis and 6 DPA and the Headline anthesis treatments resulted in lower index than the check and the Headline 6 DPA and 12 DPA treatments. FDK ranged from 41.5% in the Prosaro anthesis treatment to 79.2% in the check. It was 19% higher in the Headline anthesis treatment than in the Prosaro anthesis treatment. The Prosaro anthesis treatment reduced FDK the most followed by the Prosaro 6 DPA and Headline anthesis treatments. FDK in the 12 DPA treatments for both fungicides did not differ from that in the check. DON in all fungicide treatments was lower than that in the check. It ranged from 16.8 μg g^{-1} in the Prosaro 6 DPA treatment to 78.2 μg g^{-1} in the check. The Prosaro anthesis and 6 DPA treatments reduced DON the most. DON in the Headline anthesis and 6 DPA treatments was approximately twice that in the corresponding Prosaro treatments. TKW ranged from 19.6 g in the Headline 12 DPA treatment to 25.1 g in the Prosaro 6 DPA treatment. It was similar in the anthesis and 6 DPA treatments and higher in these treatments than in the check and 12 DPA treatments for both fungicides. Yield ranged from 988 kg ha^{-1} in the Prosaro 12 DPA treatment to 1656 kg ha $^{-1}$ in the Prosaro anthesis treatment. It was higher in the Prosaro anthesis treatment than in the Prosaro 12 DPA treatment, but did not differ among the rest of the treatments (Table 1).

Rain-fed experiment, 2016

In 2016, weather conditions were not favorable to disease development during the critical period for infection and disease progression which is from anthesis to about three weeks later. During this period, there was little rainfall and daytime temperatures were higher and RH was lower than in 2015 (Fig. 1). As a result, index, FDK, and DON were low compared to 2015 (Table 2). In the MR cultivar, index ranged from 4.5% in the Prosaro anthesis treatment to 16.7% in the check. Index in all fungicide treatments was lower than that in the check. For Prosaro, the anthesis treatment resulted in lower index than the 12 DPA treatment. For Headline, index did not differ among application timings. FDK ranged from 4.2% to 7.2% and did not differ among treatments. DON was very low at 0.1–0.2 μg g^{-1} and did not differ among treatments. TKW ranged from 22.9 g to 24.6 g and did not differ among treatments. Yield ranged from 3113 kg ha $^{-1}$ in the check to 3957 kg ha $^{-1}$ in the Prosaro anthesis treatment. For Prosaro, yield in the anthesis treatment was higher than that in the 12 DPA treatment. For Headline, yield did not differ among application timings (Table 2).

In the S cultivar, index ranged from 19.6% in the Prosaro anthesis treatment to 42.4% in the check and was higher in the Headline anthesis and 6 DPA treatments compared to the corresponding Prosaro treatments. Index did not differ among Prosaro application timings; however, for Headline, it was lower in the anthesis treatment than in the 12 DPA treatment. FDK ranged from 6.0% to 11.0% and did not differ among treatments. DON ranged from 0.4 μg g^{-1} to 1.4 μg g^{-1} and did not differ among treatments. TKW ranged from 26.5 g in the check to 34.8 g in the Prosaro 6 DPA treatment and did not differ among application timings for Headline. For Prosaro, TWK in the 6 DPA treatment was greater than that in the anthesis and 12 DPA treatments. Yield ranged from 2207 kg ha $^{-1}$ in the Headline 12 DPA treatment to 4061 kg ha $^{-1}$ in the Headline anthesis treatment. For Prosaro, the anthesis treatment resulted in higher yield than the 12 DPA treatment whereas for Headline, the anthesis treatment resulted in higher yield than the 6 DPA and 12 DPA treatments (Table 2).

Irrigated experiment, 2016

In the MR cultivar, index ranged from 16.3% in the Prosaro anthesis treatment to 38.6% in the check. It was higher in the Headline 6 DPA treatment than in the corresponding Prosaro treatment. For Prosaro, index in the anthesis and 6 DPA treatments was similar and lower than that in the 12 DPA treatment. For Headline, index in the anthesis treatment was lower than that in the 6 DPA and 12 DPA treatments. FDK ranged from 13.8% in the Prosaro anthesis treatment to 27.0% in the Headline 12 DPA treatment. For Prosaro, the anthesis treatment resulted in lower FDK than the 6 DPA and 12 DPA treatments which had similar levels. For Headline, FDK in the anthesis and 6 DPA treatments was similar and lower than that in the 12 DPA treatment. DON was low; it ranged from 0.6 μg g^{-1} to 1.2 μg g^{-1} and did not differ among treatments. TKW ranged from

17.6 g in the Prosaro 6 DPA treatment to 21.9 g in the Headline anthesis treatment. Prosaro application timings had no effect on TKW whereas for Headline, the anthesis treatment resulted in greater TKW than the 6 DPA and 12 DPA treatments. Yield ranged from 2078 kg ha $^{-1}$ in the Headline 12 DPA treatment to 2945 kg ha $^{-1}$ in the Headline anthesis treatment and did not differ among treatments.

In the S cultivar, index ranged from 26.8% in the Headline anthesis treatment to 73.5% in the check. It was higher in the Headline 12 DPA treatment than in the corresponding Prosaro treatment. For Prosaro, index in the anthesis and 6 DPA treatments was similar and lower than that in the 12 DPA treatment. For Headline, index in the anthesis treatment was lower than that in the 6 DPA treatment which was lower than that in the 12 DPA treatment. FDK ranged from 21.5% in the Prosaro anthesis treatment to 43.2% in the Headline 12 DPA treatment. It was higher in the Headline 12 DPA treatment than in the corresponding Prosaro treatment. FDK did not differ among Prosaro application timings whereas for Headline, it was lower in the anthesis and 6 DPA treatments than in the 12 DPA treatment. DON ranged from 2.6 μg g $^{-1}$ to 4.2 μg g $^{-1}$ and did not differ among treatments. TKW ranged from 25.1 g in the Headline 12 DPA treatment to 29.6 g in the Headline anthesis treatment. For Prosaro, it did not differ among application timings whereas for Headline, it was greater in the anthesis and 6 DPA treatments than in the 12 DPA treatment. Yield ranged from 1865 kg ha $^{-1}$ in the check to 3492 kg ha $^{-1}$ in the Prosaro anthesis treatment and did not differ among treatments.

Effect of environment on index, FDK, DON, and yield

Averaged over fungicide treatments and years, index and FDK were higher in the irrigated compared to the rain-fed environment in both cultivars. DON did not differ between the irrigated and rain-fed environments in the S cultivar, but was higher in the irrigated compared to the rain-fed environment in the MR cultivar. Yield did not differ between the irrigated and rain-fed environments in the S cultivar, but was lower in the irrigated compared to the rain-fed environment in the MR cultivar (Fig. 2).

Correlations among index, FDK, DON, TKW, and yield

Index and yield, index and TKW, yield and FDK, yield and DON, TWK and FDK, and TWK and DON were negatively correlated whereas index and FDK, index and DON, yield and TWK, and FDK and DON were positively correlated (Table 3). Correlations were generally stronger in the S than in the MR cultivar and in wetter, FHB-favorable (2015 rain-fed and irrigated and 2016 irrigated) environments than in a dryer (2016 rain-fed) environment. In the FHB-favorable conditions, index and DON, and index and FDK were strongly and positively correlated, which confirmed the usefulness of index as a measurement of FHB.

Discussion

Integration of cultivar resistance with other management strategies such as fungicide application, crop rotation, tillage, and irrigation management is an effective and recommended approach to managing FHB in wheat and other small grain cereal crops. In this study, Overland, a moderately resistant winter wheat cultivar, consistently had lower FHB index, FDK, and DON and yielded higher than the susceptible Overley (Tables 1 and 2). In a previous study which evaluated 363 U.S. winter wheat accessions (Jin et al. 2014), Overland was among the accessions that displayed low levels of FHB and DON, which is in agreement with the results obtained in this study. Previous research has similarly shown moderately resistant cultivars to have lower index, FDK, DON and higher yield than susceptible cultivars. Wegulo et al. (2011a) demonstrated in the field that under high FHB intensity and no fungicide application, the moderately resistant winter wheat cultivars Roane and Truman had lower FHB severity, FDK, and DON and higher yield than the susceptible cultivars Overley and Tomahawk. In a field study by Amarasinghe et al. (2013), the moderately resistant spring wheat cultivar Glenn similarly had lower FHB severity, FDK, and DON and yielded higher than the susceptible cultivar Roblin. Willyerd et al. (2012) analyzed data from more than 40 field trials conducted in 12 U.S. states and found that the wheat cultivars classified as moderately resistant, fungicide-untreated had lower index and DON compared to the cultivars classified as susceptible or moderately susceptible, fungicide-untreated.

Triazoles and strobilurins are the two classes of fungicides most widely used to control foliar and spike diseases of wheat. In this study, the triazole fungicide Prosaro was more effective in reducing index, FDK, DON, and yield loss than the strobilurin fungicide

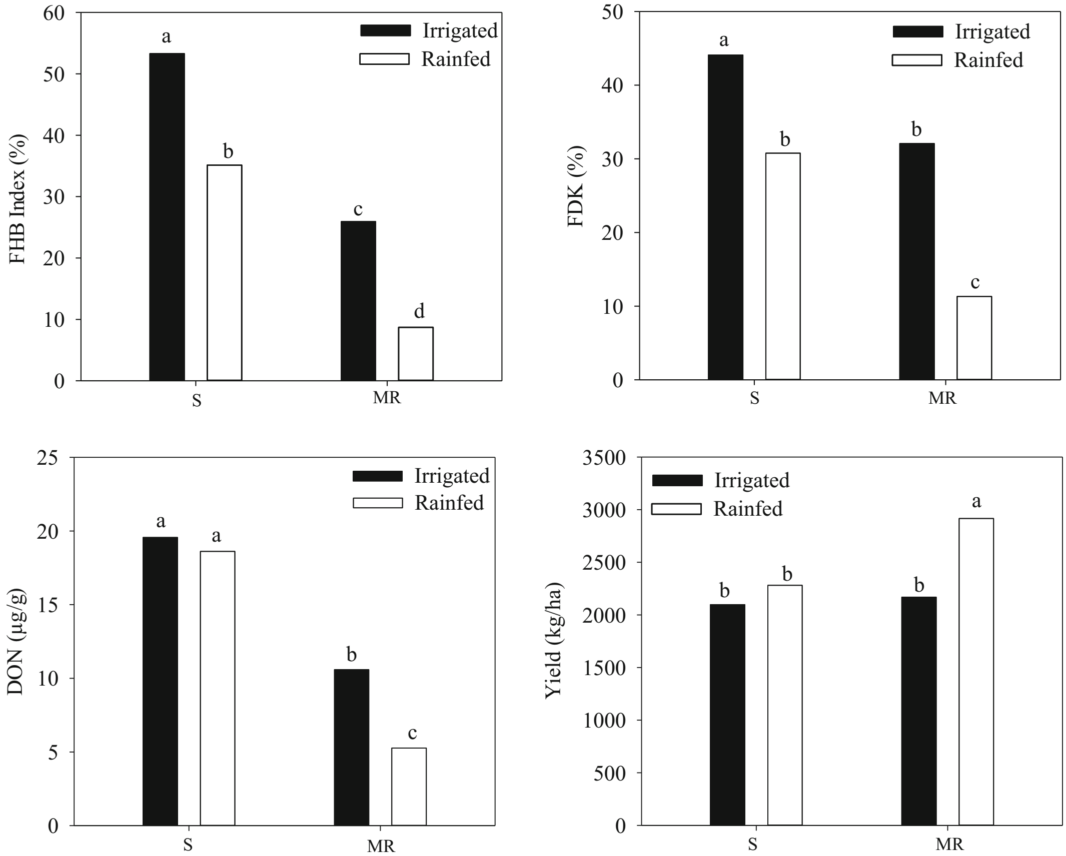

Fig. 2 Fusarium head blight index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON) concentration, and yield averaged over fungicide treatments and years to show the effect of environment on these variables in hard red winter wheat cultivars Overland (moderately resistant, MR) and Overley (susceptible, S)

Table 3 Correlation coefficients for Fusarium head blight index, yield, thousand kernel weight (TKW), *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) concentration in a moderately resistant (MR, Overland) and a susceptible (S, Overley) winter wheat cultivar. The cultivars were included in rain-fed and irrigated experiments conducted to determine the effects of fungicide chemical class, fungicide application timing, and environment on the variables during the 2015 and 2016 growing seasons

	2015				2016			
	Rain-fed		Irrigated		Rain-fed		Irrigated	
Variables	MR	S	MR	S	MR	S	MR	S
Index/Yield	−0.6498	−0.7277	−0.4450	−0.7737*	−0.8549*	−0.7893*	−0.6992	−0.8343*
Index/TKW	−0.6141	−0.6064	−0.1131	−0.8364*	−0.4050	−0.6614	−0.1351	−0.8763*
Index/FDK	0.7684*	0.8546*[a]	0.5315	0.9718**	0.2716	0.7807*	0.6695	0.8366*
Index/DON	0.6754	0.8298*	0.9545**	0.7639*	0.1372	0.5508	0.6552	0.4664
Yield/TWK	0.8124*	0.7099	0.2446	0.5455	0.3986	0.3834	0.4125	0.7310
Yield/FDK	−0.7885*	−0.9262**	−0.8236*	−0.7426	0.1151	−0.3975	−0.8356*	−0.7704*
Yield/DON	−0.5549	−0.8469*	−0.4228	−0.4760	0.1938	−0.3170	−0.5955	0.4142
TWK/FDK	−0.9382**	−0.8180*	−0.2855	−0.8362*	−0.3965	−0.5962	−0.5513	−0.8043*
TWK/DON	−0.7567*	−0.8329*	−0.2218	−0.7527	0.3741	−0.4994	−0.4788	−0.1948
FDK/DON	0.8869*	0.8525*	0.6024	0.8706*	0.5600	0.6382	0.8852*	0.7195

[a] *, **: Correlation coefficients significant at $0.01 \le P \le 0.05$, $0.001 \le P \le 0.01$, respectively

Headline. This result is consistent with results from previous studies that compared the efficacy of triazoles and strobilurins in controlling FHB and DON. Pirgozliev et al. (2002) found the strobilurin azoxystrobin to be less effective in reducing FHB and DON than the triazole metconazole. In a meta-analysis of 292 uniform fungicide field trials conducted in 17 U.S. states from 1995 to 2013, Paul et al. (2018a) found that triazoles applied to wheat at anthesis were more effective in reducing FHB and DON than strobilurins.

In addition to the inferior efficacy of strobilurins in reducing FHB and DON, this class of fungicides has been demonstrated in several studies to elevate DON. Ellner (2005) reported that in 85% of the plots in 23 field trials, strobilurins applied before anthesis (BBCH 33, 49, and 55) resulted in DON that was higher than that in untreated check plots by up to 65%. Simpson et al. (2001) and Mesterhazy et al. (2003) similarly reported elevated DON in wheat grain from field plots treated with the strobilurin azoxystrobin. In this study, the field-applied strobilurin did not result in more DON compared with the untreated check. However, when grain from the 2015 growing season was cleaned to remove FDK so it could be used in a separate study, DON in grain from strobilurin-treated plots was higher than DON in grain from untreated check plots (Bolanos-Carriel et al. 2020).

The optimum fungicide application timing to control FHB and reduce DON accumulation in grain is at early anthesis (BBCH 61) because most infections occur during anthesis or shortly thereafter (Andersen 1948). However, the short window of about 2 days during early anthesis (Barber et al. 2015) presents challenges to the grower who may not be able to spray a fungicide during that time due to various reasons including unfavorable weather, scheduling with commercial applicators, or unavailability due to personal commitments. Therefore, there have been efforts to determine whether post-anthesis fungicide applications can be effective in controlling FHB and DON. In this study, the effect of fungicide application timing was most apparent in the triazole (Prosaro) treatments applied to the S cultivar Overley in wet and humid environments, which favored high levels of FHB. In these treatments, index, DON, and FDK were similar between the anthesis and 6 DPA applications, but generally lower in the anthesis application compared to the 6 DPA application. However, index, FDK, and DON levels were significantly higher in the 12 DPA application compared to the two earlier applications.

These results suggest that fungicide application can be delayed by up to six days since early anthesis and still achieve effective control of FHB and DON. The results are similar to those obtained by D'Angelo et al. (2014) who found that index, FDK, and DON levels in post-anthesis applications of up to 6 days were generally not significantly different from those in earlier applications, although the earlier applications resulted in higher percent control compared to the untreated check. Meta-analysis of data from 19 years of fungicide field trials (Paul et al. 2018b) showed that pre-anthesis (heading) applications of the triazole fungicides Caramba® (metconazole) and Prosaro were much less efficacious in controlling FHB and reducing DON than applications at anthesis or 5 to 7 days later. The anthesis applications resulted in better control of FHB compared to applications 5 to 7 days later. However, the difference in percent control between the two timings was not large (6% and 10% for Caramba and Prosaro, respectively). For DON there was no difference in percent control between the anthesis and the late (5 to 7 days) applications (3% and 1% difference in control for Caramba and Prosaro, respectively). These results (Paul et al. 2018b) are similar to those obtained in this study and similarly suggest that a triazole application up to six days post early anthesis can control FHB and reduce DON with comparable efficacy to that achieved with anthesis applications.

The results of Paul et al. (2018b) and the results from this study indicate that applications of triazole fungicides to wheat before anthesis or too long past anthesis (12 days in this study) are not recommended because their efficacy in reducing FHB and DON is too low. In this study, efficacy of Prosaro in reducing index, FDK, and DON was highest when it was applied at early anthesis, generally decreased with each 6-day delay in application, and was lowest when the fungicide was applied 12 DPA. This suggests, as stated above, that the best window for triazole fungicide application to wheat for control of FHB and DON is early anthesis to 6 days later.

TKW and yield were reduced by both FHB and foliar diseases, most notably stripe rust and fungal leaf spots (Septoria tritici blotch and tan spot) which were present at high levels, especially stripe rust, in 2015. Because yield was reduced by both FHB and foliar fungal diseases, plots that received the delayed fungicide applications at 6 DPA and especially at 12 DPA in 2015 (disease-favorable year) had drastically lower yield than plots that were sprayed at early anthesis.

Environment played a significant role in the development of FHB and DON, which developed to higher levels in the irrigated compared to the rain-fed experiments in both years, and in 2015 when weather conditions from anthesis to three weeks later were favorable compared to 2016 when weather conditions were less favorable during the same period (Fig. 1). In 2015, weather conditions (abundant rainfall and high RH) in Nebraska's FHB-prone regions (southeast where this study was conducted, south central, and southwest) were very favorable to FHB and as a result there were major and widespread epidemics in growers' wheat fields (Lilleboe 2015). Because natural weather was already favorable for FHB development in 2015, differences in index, FDK, and DON between the rain-fed and irrigated experiments were subtle.

Index and yield, index and TKW, yield and FDK, yield and DON, TKW and FDK, and TKW and DON were negatively correlated whereas index and FDK, index and DON, FDK and DON, and yield and TKW were positively correlated. Index, FDK, and DON are all measures of the effects of FHB and are therefore expected to be positively correlated. On the other hand, because these three measures are indicators of yield loss, they are expected to be negatively correlated with yield and TKW. Yield and TKW are expected to be positively correlated because both are measures of the effects of grain fill due to accumulation of photosynthates and other products of metabolism. These results are similar to those reported by Menniti et al. (2003), Wegulo et al. (2011 a, b), and Hernandez-Nopsa et al. (2012). Correlations were generally stronger in the S than in the MR cultivar. Wegulo et al. (2011) similarly found that there was a stronger relationship between index and DON, index and FDK, and DON and FDK in susceptible compared to moderately resistant winter wheat cultivars. Correlations were also generally stronger in FHB-favorable environments (2015 growing season and irrigated experiments) than in a relatively dry environment (2016 rain-fed experiment). This observation is attributable to greater differences among fungicide treatments in susceptible compared to moderately resistant cultivars and in disease-favorable compared to disease-unfavorable environments. The strong correlation between index and DON, and index and FDK in FHB-favorable conditions (wet environments and the S cultivar) indicated that index is a useful and reliable measurement of FHB.

This study confirmed, under Nebraska field conditions, that a triazole fungicide was more effective than a strobilurin fungicide in reducing FHB index, FDK, and DON compared to a triazole fungicide. In addition, the study showed that early anthesis applications of a triazole fungicide were the most effective in reducing index, FDK, and DON, but applications at 6 DPA had comparable efficacy, indicating that growers have a wider window during which they can apply a triazole fungicide to control FHB and DON during disease-favorable growing seasons. We recommend to growers the planting of moderately resistant cultivars. In FHB-favorable (wet and humid) growing seasons, we recommend the application a triazole fungicide during the six-day window starting at early anthesis to both moderately resistant and susceptible cultivars. Even when FHB intensity is high, which reduces fungicide efficacy and overwhelms the resistance in moderately resistant cultivars, applying a triazole fungicide at anthesis significantly reduces index, FDK, and DON, which in turn reduces discounts in the price of grain at the point of sale.

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Compliance with ethical standards The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The research reported in this paper did not involve human participants or animals. This paper is currently submitted only to the European Journal of Plant Pathology. The research reported in this paper is original research conducted to demonstrate the effects of cultivar resistance, fungicide chemical class, and fungicide application timing on Fusarium head blight in winter wheat under Nebraska field conditions.

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