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Fungicide application at anthesis of wheat provides effective control of leaf spotting diseases in western Canada

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ABSTRACT

Leaf spotting diseases commonly occur on spring wheat crops grown in Saskatchewan, causing yield losses of up to 15%, although greater losses have been documented during severe epidemics. In the past decade, Fusarium head blight (FHB) has become a major concern for growers resulting in extensive use of fungicide to mitigate the disease. The optimal fungicide timing for leaf spot control is generally at the flag-leaf stage, while the optimal timing for FHB is during anthesis. The objective of this study was to determine whether applying fungicide at anthesis timing can provide adequate control of leaf spots when compared to application at flag-leaf stage. Fungicide treatments applied at flag-leaf, anthesis, and both growth stages were evaluated on the cv. Carberry. Prothioconazole + tebuconazole and tebuconazole (only) applied at anthesis provided adequate control of leaf spots, although the severity of leaf diseases was slightly higher in this treatment than application at flag-leaf stage but yields were similar. Test weight and thousand kernel weight were improved with the application at anthesis relative to that at flag-leaf stage. Two applications of fungicide provided only a small incremental benefit to the anthesis application, and would not be economically justified in western Canada at this time. Anthesis fungicide application provided adequate leaf spot disease control and is the optimum timing for the control of FHB.

1. Introduction

Fungicide application timing is critical for effective control of fungal plant diseases. In cereal crops such as wheat and barley, the flag and penultimate leaves are major contributors to grain yield and quality (Bhathal et al., 2003) and therefore fungicides are used to protect these leaves from leaf spotting diseases. Previous work has proven that very early application of fungicide at the seedling stage (BBCH 12–14, Lancashire et al., 1991) in barley to mitigate leaf spotting diseases provides very little to no improvement in yield and quality (Turkington et al., 2015). In recent years, wheat growers in western Canada have applied fungicide at the anthesis stage (BBCH 61–65), instead of, or in addition to application at flag leaf stage (BBCH 39). The anthesis stage is the recommended time for fungicide application to suppress fusarium head blight (FHB) caused by *Fusarium graminearum* Schwabe *sensu lato* (syn. *Gibberella zeae* (Schwein.) Petch), but the effectiveness of fungicide applied at this timing against leaf spotting diseases is unknown.

Fusarium head blight is a major disease of cereals that has become common over the past two decades across the Canadian Prairies and the Pacific northwest of the United States. The disease results in the premature senescence of the entire spike or a portion of the spike, causing a reduction in yield and grain quality (Haidukowski et al., 2005). Yield losses of over 70% have been reported when FHB reaches epidemic levels. *Fusarium graminearum* can also produce a wide assortment of mycotoxins, the most common of which is deoxynivalenol (DON) (Bottalico and Perrone, 2002). DON is neurotoxic and immunotoxic and even a low concentration can have detrimental effects on both humans and livestock (FAO/WHO, 2001). Several European countries have placed limits on the levels of DON allowable in wheat and cereal products intended for human consumption. These limits are 2.0 mg kg^{-1} for wheat and unprocessed wheat products, 1.0 mg kg^{-1} for wheat

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flour, pasta, and bakery products, and 0.5 mg kg^{-1} for food products intended for infants and young children (Koornneef et al., 2002). The allowable DON limits in Canadian wheat are 2.0 mg kg^{-1} for uncleaned soft wheat for use in non-staple foods and 1.0 mg kg^{-1} for uncleaned soft wheat for use in baby foods (Haidukowski et al., 2005). Due to the serious consequences of this disease, many wheat growers in western Canada have adopted fungicides as part of an integrated strategy for FHB management.

There are several leaf spotting diseases that affect spring wheat, including tan spot, caused by *Pyrenophora tritici-repentis* (Died.) Drechsler and multiple pathogen species that constitute the septoria leaf spot complex. These include *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (syns. *Mycosphaerella graminicola* (Fuckel) J. Schröt.; *Septoria tritici* Desm., causal agent of septoria tritici blotch, as well as *Parastagonospora nodorum* (Berk.) Quaedvlieg, Verkley, & Crous and *Parastagonospora avenae* (A. B. Frank) Quaedvlieg, Verkley, & Crous, causal agents of stagonospora blotch (Goodwin, 2012; Murray et al., 2015). Tan spot may lead to yield losses of 50–75% in wheat in severe cases (Hosford, 1971; Hosford and Busch, 1974; Rees and Platz, 1980) and yield losses of 30–50% have been attributed to septoria leaf blotch during severe epidemics (Eyal et al., 1987). Stagonospora blotch infection generally leads to minor yield loss, most often resulting in 10–15% lower production overall (King et al., 1983). The environment and cultural practices play large roles in the severity of leaf spotting diseases, hence resulting in variable impact on yield depending on the year and location. For instance, Evans et al. (1999) reported that leaf spots reduced wheat yield in inoculated plots by 15% in Oklahoma, which was similar to the findings of Shabeer and Bockus (1988) in Kansas. Rees et al. (1982), however, reported wheat yield losses of nearly 50% in fungicide untreated plots in Australia.

Fungicides are strategically applied to protect the flag leaf from leaf spot pathogen infection (Wegulo et al., 2012), because most fungicides are ineffective once leaf spot symptoms have appeared (McGrath, 2004). Protecting the flag leaf is paramount because it is responsible for a large portion of grain filling in wheat (Ruske et al., 2003; Simpson, 1968). The optimal time to apply fungicide in wheat has been contested, although several studies have suggested that early fungicide application, at flag leaf stage, is imperative to improve yield (Wegulo et al., 2012). Optimal fungicide timing to control FHB has been suggested to be at the anthesis stage (Halley et al., 2001); however, the optimal timing to control leaf spots has been reported to be at the flag leaf stage (Wiersma and Motteberg, 2005). In durum wheat in Saskatchewan, fungicide application at the flag leaf stage reduced leaf spotting diseases on the flag and penultimate leaves more than application at anthesis, as determined from disease assessment at the early milk stage; however, when leaf diseases were measured at the soft dough stage an application at both flag leaf and anthesis stages resulted in the lowest disease severity and highest grain yield (May et al., 2014).

Prothioconazole + tebuconazole (Prosaro®) has been recommended to control FHB, as it has been shown to be more effective than tebuconazole alone (Folicur® 432F; Folicur 250EW; Palliser®; Fuse®; Hornet® 432F) at reducing DON (Paul et al., 2007). Before the availability of prothioconazole, experiments conducted in both Minnesota and North Dakota indicated that tebuconazole was the most effective of the triazole fungicides (McMullen et al., 1997; McMullen, 1998). Serenade® Optimum is a bio-fungicide made from the QST 713 strain of *Bacillus subtilis* (Bayer Crop Science), a gram-positive endospore-forming bacterium that can produce more than two dozen antibiotics (Moszer et al., 2002). Serenade Optimum is the only Group 44 fungicide and possesses a unique mode of action making it difficult for pathogens to develop resistance (Bayer Crop Science). However, there are no studies on the effect of this product on leaf spotting diseases of wheat from western Canada and it is not currently registered for use on wheat.

The objective of this study was to assess whether applying fungicide at anthesis stage (BBCH 61 to 65), the recommended timing for FHB management would adequately control leaf spots in spring wheat compared to an application at flag leaf stage (BBCH 39), while providing economic benefits to growers.

2. Materials and methods

2.1. Study area

The study was conducted at Saskatoon (52.1332°N, 106.6700°W, Black soil zone), Indian Head (50.5334°N, 103.6699°W, black soil zone), and Melfort (52.8608°N, 104.6143°W, Black soil zone) in Saskatchewan, as well as Lethbridge (49.6935°N, 112.8418°W, Brown soil zone) and Brooks (50.5334°N, 111.8992°W, Brown soil zone) in Alberta in 2013. The study was repeated in 2014 at Saskatoon and Melfort, SK, and at Lethbridge, Brooks and Lacombe (52.4631°N, 113.7286°W, Black soil zone), Alberta. In 2015, the locations were Saskatoon, Indian Head, Melfort, Brooks and Lethbridge, while Lacombe was lost to hail. Plots were established at Saskatoon in a field previously sown to canola in 2012 and wheat in 2013, at Indian Head in a field previously sown to wheat in 2012, at Melfort in a field previously sown to canola in 2012 and 2013, at Brooks in a field previously sown to alfalfa in 2012 and summer fallowed in 2013, at Lethbridge in a field previously sown to barley in 2012 and 2013, and at Lacombe in a field previously sown to wheat in 2013. At all sites, glyphosate (900 g a. i. ha^{-1}) was applied to the entire plot area prior to seeding to suppress weeds. Subsequent herbicide treatments were applied at recommended rates and timings following Guide to Crop Protection (www.saskatchewan.ca/agrcuture) to control weeds as necessary at each site. Soil samples were collected at each site in early spring and fertilizer applied to achieve 100% of the soil test recommendations. Precipitation and temperature values were recorded at each site-year for the growing period from April until August and were compared to the long-term climate normal calculated from data over the period 1981 to 2011 (Table 1).

2.2. Experimental design

The plot sizes were 2 × 8 m at Saskatoon, 4 × 10.7 m at Indian Head, 4 × 10 m at Melfort, 1.2 × 6 m at Brooks, 2 × 6 m at Lethbridge and 1 × 5.5 m at Lacombe. Seeding depth at each site varied from 3.81 cm to 7.62 cm, while row spacing varied from 20.3 to 30.5 cm and seeding rate from 250 to 275 seeds m^2. Experiments were designed as randomized complete blocks of four replicates. Each trial consisted of a 3 × 3 factorial arrangement plus an unsprayed check, for a total of 10 treatments. Three fungicide application timing treatments consisted of a single application at flag leaf stage (ZGS39), a single application at anthesis (ZGS60) and a dual application at each ZGS39 and ZGS60. The fungicide treatments were: 1) prothioconazole + tebuconazole (Prosaro at 800 mL ha^{-1}), 2) tebuconazole (Folicur 250 EW at 499 mL ha^{-1}, or at Lacombe, 2014; Folicur 432 F at 291 mL ha^{-1}), 3) *B. subtilis* (Serenade Optimum 500 g ha^{-1}), all applied in 100 L ha^{-1} of water.

The Canada Western Red Spring wheat variety 'Carberry' was chosen for this study as it is moderately susceptible to leaf spotting diseases, but moderately resistant to FHB (Anon, 2014). This choice of variety was made to help reduce the confounding effects between leaf spots and FHB. Disease ratings were conducted at the soft dough stage (ZGS 85). Leaf spotting disease severity was determined by assessing ten flag leaves and ten penultimate leaves from each plot using the Horsfall–Barratt (0–11) scale (Horsfall and Barratt, 1945). Ratings were then converted to percent leaf area affected by the disease according to the grade formula specified in the scale. Fifty random spikes were assessed from each plot at the early dough stage of development for FHB severity (proportion of the spike bleached) by taking the average disease severity (%) of the 50 heads collected using the scale of Stack and McMullen (1995). Wheat was harvested from a 10–42 m^2 area of each plot, excluding outer row on each side of each plot, using a small-plot

Table 1
Summary of climatic conditions for experimental sites in Alberta and Saskatchewan in 2013–2015. The 30-year averages were long-term climate normal from Environment Canada weather stations (1981–2011) at or close to the sites.

Location/year	Precipitation (mm)					5-mo total	% of 30-yr average	Temperature (°C)					% of 30-yr average
	Apr.	May	June	July	Aug.			Apr.	May	June	July	Aug.	
Saskatoon													
2013	10.5	15.9	117.7	35.6	14.9	194.6	83	−2.3	13	15.5	17.4	18.9	88
2014	74.2	61.1	94.8	44.5	18.5	293.1	125	1.7	10.1	14.1	18.3	17.9	87
2015	21.1	0.4	13.6	84.3	45.2	164.6	70	5.6	10.1	17.2	19.4	17.4	98
30-yr average	18	50	60	63	43	234		5.3	12.2	16.6	19.1	18	
Melfort													
2013	5.8	19	97.9	103.2	11.7	237.6	96	−3.9	12	15.4	16.4	17.7	93
2014	50.3	24.3	167.3	38.8	57.9	338.6	137	−1	10	14	17.5	17.6	93
2015	34.4	7.1	54.8	149.8	57.4	303.5	123	3.8	9.9	16.4	17.9	17	105
30-yr average	20	47	70	77	33	247		3.1	9.6	14.9	17.3	17.3	
Indian Head													
2013	7.6	18	105	50.9	7.1	188.6	70	−4.6	11.9	15.3	16.3	17.1	83
2015	9.5	15.6	38.3	94.6	58.8	216.8	81	4.8	10	16.2	18.1	17	98
30-yr average	16	53	79	67	53	268		4	11.4	16.1	18.4	17.5	
Brooks													
2013	23.1	38.9	92.2	53.7	17.3	225.2	132	2.8	12.5	15.4	17.5	18.8	94
2014	21.9	29.6	93.2	33.7	29.8	208.2	122	5.1	10.3	14	19.5	18.5	94
2015	1.8	4.6	20.8	16.4	47.4	91	53	6.3	10.4	17.2	18.9	18.2	99
30-yr average	12	35	58	32	34	171		6.2	11.4	15.8	19.2	18.9	
Lethbridge													
2013	9.5	81.9	82	49.9	35	258.3	129	3.2	11.3	14.3	17.4	18.3	93
2014	29.6	38.1	49.5	16.7	49	182.9	91	4.8	10.1	13.5	18.5	17.5	93
2015	0	29.3	13.4	39.3	16.1	98.1	49	5.9	9.4	16.9	18.3	18.2	100
30-yr average	17	46	53	37	47	200		6.1	11.4	15.6	18.2	17.7	
Lacombe													
2014	22.4	45	83.2	72.6	24.7	247.9	73	2.8	8.9	13.3	17.4	15.7	100
30-yr average	17	45	92	117	67	338		4	9.3	13.1	16	15.7	

combine. Samples were air-dried for approximately 48 h, adjusted to 14.5% moisture content and cleaned. Harvest data included plot yield (kg ha^{-1}), test weight (TW; kg hL^{-1}), and thousand kernel weight (TKW; g). A subsample from the yield sample of each plot was analyzed to determine % protein content (at 12.5% moisture content) using a LECO protein analyser (LECO corporation®).

The data collected from each site-year were classified into high disease locations, where leaf-spot disease severity was 40% or greater in unsprayed checks, and low disease locations, where disease severity was less than 40%. Data from high disease site-years was combined, as was data from low disease site-years, and each analyzed separately. High disease locations included Saskatoon, Melfort and Lacombe in 2014, while all other site-years were considered low disease locations.

2.3. Data analysis

All statistical analyses were performed using the mixed procedure of SAS version 9.3 statistical software (SAS Institute Inc., Cary, NC). The treatments were considered fixed effects, and location and year, as well as blocks within location*year were considered random effects. The DDFM = kenwardroger option was considered for approximating the degrees of freedom for means. Contrast statements were used to make comparisons among treatments of interest, which were accepted as significantly different at ($P \leq 0.05$).

3. Results

Monthly accumulated precipitation and average temperature from April 1st to August 31st for each year and location are provided in Table 1. Precipitation was between 49 and 137% of the long-term average at six locations in 2013, 2014, and 2015. Two of the three high disease severity locations had two of the highest precipitation values, Saskatoon in 2014 had 293 mm, 125% of the 30-yr average and Melfort in 2014 had 339 mm, 137% of the 30-yr average. Lacombe in 2014 was a high leaf-spot disease severity location although precipitation was only 248 mm, 73% of the 30-yr average. It should also be noted that Saskatoon in 2014 and Melfort in 2014 had the highest amount of rainfall in April compared with all other locations and years at 74 mm and 50 mm respectively and were also two of the coldest locations in April, with average temperatures of 1.7 °C and -1 °C. However, Lacombe in 2014 had low average precipitation at 22 mm in April, although temperatures in April were also relatively cold, averaging 2.8 °C, which was colder than the majority of the other locations and years. At high leaf-spot severity site-years treatment effects were detected for leaf spot disease severity, yield, TW and TKW, but not FHB severity or PC, while at low leaf-spot disease severity site-years only leaf spot disease severity and TKW were significant (Table 2).

3.1. High leaf-spot severity site-years

The mean leaf-spot disease severity of the unsprayed check was 73.2%, which was reduced to 32.0% by fungicide application (average of the three treatments; Table 3). There were differences in disease severity among fungicide treatments with the highest severity observed by a single fungicide application at anthesis (41.0%), the lowest with the dual application (20.2%), and intermediate severity for the flag leaf application (34.7%). Only the synthetic fungicides reduced leaf-spot disease severity compared to the untreated control; the biological fungicide (*B. subtilis*) did not. Overall, tebuconazole reduced severity to 36.1%, and prothioconazole + tebuconazole to 27.6%. The FHB severity was ≤4.0% and no differences were observed among treatments (Table 3).

Yield was increased by 12.4% with synthetic fungicide application (average of the three fungicide treatments) for the high disease site-years (Table 3); the unsprayed check had a mean yield of 3676 kg ha^{-1} and the fungicide treatments 4132 kg ha^{-1}. Yield of the flag leaf treatment (4010 kg ha^{-1}) was less than the dual application treatment (4246 kg ha^{-1}). There was no difference between the flag leaf and anthesis treatments or the anthesis treatment and the dual application treatment. Yield differences were not detected between the synthetic

Table 2

The *F* and *P* values from the analysis of variance for the fungicide treatment effect on leaf spot disease severity (LSDS), Fusarium head blight (FHB) severity, yield, test weight (TW), thousand kernel weight (TKW), and protein content (PC) at high (Melfort, Saskatoon, and Lacombe in 2014) and low (Saskatoon, Melfort, Indian Head, and Brooks in 2013; Lethbridge in 2013 and 2014; Saskatoon, Indian Head, Melfort, Brooks, and Lethbridge in 2015) disease severity locations.

Treatment/Contrast	LSDS		FHB		Yield		TW		TKW		PC	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
High disease severity locations	30.94	< 0.0001	0.76	0.7199	5.23	< 0.0001	4.45	< 0.0001	15.83	< 0.0001	1.08	0.3971[a]
Low disease severity locations	22.65	< 0.0001[b]	1.43	0.4306	1.20	0.2638	1.02	0.4300[c]	3.07	< 0.0001	1.43	0.1274[d]

[a] Only data from Saskatoon in 2014 were analyzed.

[b] Data were square-root transformed; data from Brooks in 2013 and 2015, and Lethbridge in 2014 were not included.

[c] Data from Brooks in 2013 were not included.

[d] Data from Brooks, Indian Head, and Lethbridge in 2013 were not included.

fungicides. There was a yield decrease of 9.1% with the biological fungicide (3346 kg ha^{-1}) compared with the unsprayed check (3676 kg ha^{-1}).

There was a slight difference in TW between the unsprayed check (78.2 kg hL^{-1}) and the fungicide treatments (78.5 kg hL^{-1}) (Table 3). There was also a difference between the flag leaf treatment (78.3 kg hL^{-1}) compared to the anthesis treatment (78.6 kg hL^{-1}) or the dual application treatment (78.6 kg hL^{-1}). Thousand kernel weight differed between the unsprayed check (33.4 g) and the fungicide treatments (36.0 g). There were also slight, but significant differences in TKW among the fungicide treatments: flag leaf stage (35.4 g); anthesis timing (36.0 g); and dual (36.6 g). Between fungicide treatments, TKW was higher for prothioconazole + tebuconazole (36.3 g) relative to tebuconazole alone (35.8 g). There were no differences among fungicide treatments or between any of the fungicide treatments and the unsprayed check with respect to protein content. Additionally, there was no effect of the biological fungicide on any of the seed quality factors measured.

Yield was negatively correlated with leaf-spot disease severity, but positively correlated with TW and TKW at high disease severity locations (Fig. 1). Leaf-spot disease severity was inversely correlated with TW and TKW and TW and TKW were positively correlated.

3.2. Low disease severity site-years

The mean leaf spot disease severity of the unsprayed check at the low disease locations was 16.2%, which was reduced to 6.6% with the application of fungicide (Table 4). Leaf spot severity was slightly lower in the dual fungicide treatment (5.0%) compared to application at either flag leaf (7.1%) or anthesis (7.6%). The FHB severity was similar to the high disease severity site-years (≤3.9%) and no differences were detected among treatments. Thousand kernel weight was also lower when fungicide was applied at flag leaf stage (38.7 g) compared to anthesis stage (39.1 g) or to the dual fungicide treatment (39.3 g), while the anthesis and dual treatments did not differ. There were no differences in yield, TW or protein content among any of the treatments (Table 4).

4. Discussion

It is important to manage FHB and leaf spot diseases in wheat to reduce their impact on yield and quality. Previous studies of fungicide timing on wheat have been inconsistent, with variable conclusions on when to apply fungicide for optimal leaf spot control. Our study attempted to determine the optimal time and frequency to apply fungicide for control of both leaf-spot diseases and FHB in spring wheat in western Canada.

Differences in leaf disease severity between flag leaf and anthesis stage fungicide applications were only detected at the high disease locations, where applying fungicide at flag leaf stage reduced leaf disease by only 6% relative to fungicide application at anthesis. Differences in leaf disease severity were also observed when fungicide was applied at flag leaf or anthesis timings compared to the dual application treatment under both high and low disease severity situations. Our results are in agreement with Duczek and Jones-Flory (1994), who claimed the optimum time to apply fungicide was between the flag leaf and the

Table 3

Means and standard errors of fungicide product and timing treatments for leaf disease severity (LSDS), fusarium head blight severity (FHB), yield, test weight (TW), thousand kernel weight (TKW) and protein content (PC); and *P* values for select contrasts at high disease severity locations (Melfort, Saskatoon and Lacombe in 2014).

Treatment/Contrast	LSDS (%)	FHB (%)	Yield (kg ha^{-1})	TW (kg hL^{-1})	TKW (g)	PC (%)
Fungicide Timing Treatments						
unsprayed control	73.2 ± 4.1	3.2 ± 1.0	3676 ± 233.6	78.2 ± 0.6	33.4 ± 0.4	14.8 ± 0.2
flag leaf	34.7 ± 2.5	3.6 ± 0.6	4010 ± 163.0	78.3 ± 0.3	35.4 ± 0.3	15.3 ± 0.3
anthesis	41.0 ± 3.3	4.0 ± 0.6	4141 ± 131.6	78.6 ± 0.3	36.0 ± 0.3	15.2 ± 0.2
both timings	20.2 ± 2.3	3.1 ± 0.6	4246 ± 156.1	78.6 ± 0.3	36.6 ± 0.3	15.5 ± 0.2
Fungicide Product Treatments						
B. subtilis	68.9 ± 3.2	3.4 ± 0.6	3346 ± 141.0	78.0 ± 0.3	33.3 ± 0.3	15.0 ± 0.2
fungicides (combined)	31.8 ± 1.7	3.6 ± 0.3	4133 ± 87.0	78.5 ± 0.2	36.0 ± 0.2	15.3 ± 0.1
prothioconazole + tebuconazole	27.6 ± 2.4	3.9 ± 0.5	4136 ± 130.1	78.5 ± 0.3	36.3 ± 0.2	15.3 ± 0.2
tebuconazole	36.1 ± 2.4	3.2 ± 0.4	4130 ± 116.5	78.5 ± 0.2	35.7 ± 0.2	15.4 ± 0.2
flag vs. anthesis	0.0198	0.6315	0.2343	0.0008	0.0156	0.6627
flag vs. both	< 0.0001	0.3829	0.0267	0.0001	< 0.0001	0.3994
anthesis vs. both	< 0.0001	0.1771	0.2987	0.5788	0.0277	0.2037
unsprayed vs. biological	0.2861	0.9270	0.0022	0.2135	0.8835	0.5482
unsprayed vs. fungicide	< 0.0001	0.7139	0.0044	0.0139	< 0.0001	0.1201
prothioconazole + tebuconazole vs. tebuconazole	< 0.0001	0.1201	0.9470	0.7338	0.0033	0.7384

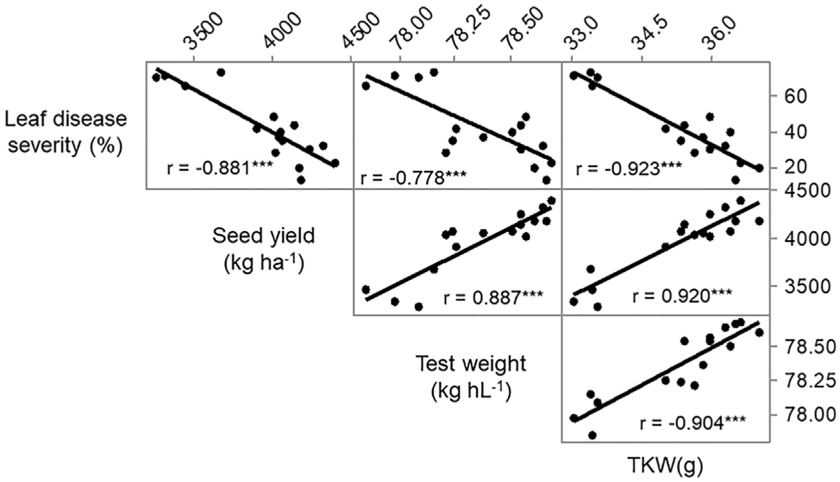

Fig. 1. Correlation matrix among leaf disease severity (%), yield (kg ha^{-1}), test weight (kg hl^{-1}), and thousand kernel weight (TKW; g) at high disease severity locations (Melfort, Saskatoon, and Lacombe in 2014).

medium milk growth stages. Wiersma and Motteberg (2005) also reported similar results, that applying fungicide at flag leaf stage and anthesis gave similar leaf spot disease control in two of three years, while in one year application at anthesis provided greater disease control than application at flag leaf stage. Similarly, Bockus et al. (1997) reported that the optimum disease control occurred between the boot and fully headed growth stages.

Fungicide application in wheat has been reported to dramatically reduce leaf spot disease severity, for example from 81.5 to 10.9% (Ransom and McMullen, 2008). The most effective treatment in our study was the dual application of fungicide, which reduced leaf disease severity from 73.2% in the unsprayed check to 20.2% at the high disease locations, and from 13.7% to 6.0% the low disease locations.

Application of fungicide when the flag leaf is first visible to soft red winter wheat in the eastern United States (Indiana, Wisconsin, Illinois, and Ohio) reduced the risk of septoria leaf blotch reaching the flag leaf by 55–75% compared to a non-treated control, whereas application at flag leaf stage, reduced risk by only 62–69% (Willyerd et al., 2015). In that study, a split application of a half-rate of fungicide at the seedling stage (three leaves unfolded) and at flag leaf first visible reduced the risk of septoria leaf blotch reaching the flag leaf by 67–70%. They also noted that applying fungicide only at the seedling stage reduced the risk of septoria leaf blotch reaching the flag leaf between 32 and 37%. However, they did report that the split-rate application at both of these growth stages provided a higher yield response than a single application at either stage. This would suggest, counter to our results and the results of several earlier studies, that a split application with the first application at an early growth stage, is important to control leaf-spot diseases. Our study has no equivalent treatment to compare with the above study, but it was determined that dual fungicide application (at both flag leaf and anthesis stages) would provide greater disease control and higher yields than applying fungicide at a single application timing.

Applying the same fungicide family twice per growing season is discouraged due to increased selection for fungicide insensitive pathogen isolates. It was noted by Chang et al. (2007) that applying pyraclostrobin several times each year led to the selection of fungicide insensitive pathogen populations, while also contributing to the reduction in efficacy of pyraclostrobin to control ascochyta blight. As for application timing, applying fungicide over a wide range of growth stages should be examined to select the most effective timing for optimal disease control and crop yield; however, opinions on the optimum fungicide timing vary. Lopez et al. (2015) believe that the optimum

Table 4

Means and standard errors of fungicide product and timing treatments for leaf disease severity (LSDS), fusarium head blight severity (FHB), yield, test weight (TW), thousand kernel weight (TKW) and protein content (PC); and P values for select contrasts for harvest data at low disease locations (Saskatoon, Melfort, Indian Head and Brooks in 2013, Lethbridge in 2013 and 2014 and Saskatoon, Indian Head, Melfort, Brooks and Lethbridge in 2015).

Treatment/Contrast	LSDS (%)	FHB (%)	Yield (kg ha^{-1})	TW (kg hL^{-1})	TKW (g)	PC (%)
Fungicide Timing Treatments						
unsprayed control	16.2 ± 2.2	3.8 ± 1.7	4413 ± 159.2	77.3 ± 1.2	38.6 ± 0.4	14.9 ± 0.3
flag leaf	7.1 ± 0.4	3.8 ± 0.9	4417 ± 77.4	76.7 ± 0.6	38.7 ± 0.3	15.1 ± 0.1
anthesis	7.6 ± 0.5	3.4 ± 0.8	4397 ± 77.3	77.1 ± 0.6	39.1 ± 0.2	15.1 ± 0.2
both timings	5.0 ± 0.2	3.7 ± 0.9	4435 ± 75.1	76.9 ± 0.6	39.3 ± 0.2	15.2 ± 0.1
Fungicide Product Treatments						
B. subtilis	14.9 ± 1.1	3.9 ± 1.1	4349 ± 88.5	76.8 ± 0.7	38.5 ± 0.2	15.0 ± 0.2
fungicides (combined)	6.6 ± 0.2	3.6 ± 0.5	4414 ± 44.1	76.9 ± 0.3	39.0 ± 0.1	15.1 ± 0.1
prothioconazole + tebuconazole	6.1 ± 0.3	3.7 ± 0.7	4426 ± 62.9	77.0 ± 0.5	39.0 ± 0.2	15.1 ± 0.1
tebuconazole	7.0 ± 0.4	3.5 ± 0.7	4403 ± 62.1	76.8 ± 0.5	39.0 ± 0.2	15.1 ± 0.1
flag vs. anthesis	0.4497	0.1714	0.5468	0.1967	0.0008	0.7361
flag vs. both	0.0020	0.7430	0.7617	0.6452	< 0.0001	0.3480
anthesis vs. both	0.0001	0.2982	0.3656	0.4059	0.3059	0.2026
unsprayed vs. biological	0.2193	0.8440	0.7598	0.9919	0.8249	0.3437
unsprayed vs. fungicide	< 0.0001	0.5998	0.9956	0.3702	0.0322	0.0710
prothioconazole + tebuconazole vs. tebuconazole	0.1007	0.5029	0.5640	0.5074	0.8960	0.5047

timing should be considered in relation to disease development, but Paveley et al. (1997) suggest that disease severity is an inconsistent measure for determining yield loss, even when multiple ratings are taken at various growth stages. In the current study, we took the importance of both leaf-spot disease and FHB control into consideration and assessed the potential compromise in fungicide application timing for optimal yield, grain quality and economic benefit.

Differences were observed in leaf disease severity between prothioconazole + tebuconazole and tebuconazole alone; the former was 23.5% more effective in high-disease fields and 13.8% in low-disease fields, although no differences in yield were observed. Jørgensen and Thygesen (2006) suggest that based on disease control levels observed at early milk and late milk, strobilurins such as pyraclostrobin, picoxystrobin and azoxystrobin provide greater control of tan spot than triazoles. Willyerd et al. (2015), however, reported no yield difference from plots treated with pyraclostrobin or prothioconazole + tebuconazole. Lopez et al. (2015) found that propiconazole and prothioconazole were the most effective triazoles for leaf-spot disease control in wheat. This may explain the results of our study where prothioconazole + tebuconazole provided slightly better disease control than tebuconazole alone, due mostly to the higher efficacy of prothioconazole.

Fungicide applied at flag leaf stage and again at anthesis (the dual treatment) reduced disease severity compared to a single application at either stage. Fungicide applied at both stages increased yield compared to a fungicide application flag leaf stage; however, the increase in yield over a single application at flag leaf stage was marginal and the limited yield increase compared to a single application at anthesis would not justify a second application economically. Similar findings were reported by Wegulo et al. (2009) who determined that tan spot disease symptoms during flowering had the strongest relationship with yield, relative to the seven other growth stages at which disease was assessed. Our observations also agree with those of Shaner and Buechley (1995) in Indiana over a 19-year period, and by Milus and Chalkley (1997) on plots infected with *P. nodorum*. These studies indicated that applying fungicide before flag leaf stage is unnecessary. In our study, a single fungicide application at anthesis was the optimal timing for leaf-spot control, grain yield and grain quality.

The bio-fungicide did not affect disease severity, TW, TKW or protein content compared to the unsprayed check under either high or low disease conditions (Tables 3 and 4). However, yield was reduced by 9.1% in high-disease locations, and we have no logical explanation for this.

Fusarium head blight severity was low in our study and was not reduced further by the biological or synthetic fungicides in either the high or low leaf-spot disease site-years. Lopez et al. (2015) reported similar results, noting that FHB severity was only marginally reduced with application of epoxiconazole + pyraclostrobin.

The average yield increase due to fungicide treatment (single or dual) over the control in this study was 12.4%. In a durum wheat study conducted in Manitoba and Saskatchewan with high leaf spot disease severity, a dual application (at flag leaf stage and anthesis stages), reduced leaf spot severity and resulted in an 8% yield increase (May et al., 2014), similar to our study. These yield increases were much lower than reported by Wiersma and Motteberg (2005), who noted a 42% increase, when trifloxystrobin + propiconazole was applied at early (first node visible) and at flag leaf stage on hard red spring wheat in Minnesota between 2001 and 2003. Ransom and McMullen (2008) also found that strobilurin fungicides improved yield between 5.5 and 44% in North Dakota, and Lopez et al. (2015) noted an increase in yield between 800 and 1690 $\mathrm{kg\,ha^{-1}}$, depending on the fungicide applied and suggested that two- or three-spray strategies would provide the greatest yield increases. Fungicides reduced leaf disease and increased yield to a greater degree in these studies relative to what was observed in our study, which may be because these studies were conducted in environments more favorable for crop and disease development.

High TW and TKW are indicative of high grain quality. Although disease severity was slightly higher with fungicide application at anthesis stage compared with flag leaf stage, yield did not differ between the two treatments. This may be because applying fungicide at anthesis can result in higher TW and TKW due to the impact of disease development on the tissues of the spikes, which contribute to grain filling and yield. The development of FHB was not affected by the various treatments and thus was not a factor influencing grain filling and yield. When leaf spot severity was high, TW and TKW were improved by fungicide treatment compared with the unsprayed check. For the high disease locations, the application of prothioconazole + tebuconazole resulted in higher TKW compared with tebuconazole alone, but not for TW. Fungicide application had a beneficial effect on TW and TKW, as reported previously by Wiersma and Motteberg (2005). Reduced leaf disease severity results in a greater photosynthetic area, thus increasing grain filling and therefore TKW. For TW, the improvement was greater when fungicide was applied at the anthesis stage relative to the flag-leaf stage. Fungicide application had no impact on protein content at either high or low leaf spot severity site years in our study. Ruske et al. (2003) made similar observations and suggested that fungicide application would not lead to dilution of protein. We conclude that delaying fungicide application from the flag-leaf stage to anthesis will not have negative effects on grain protein content.

From the current study, there was no yield penalty when applying fungicide at the anthesis stage compared to flag-leaf stage for control of leaf spotting diseases under high disease scenarios. Our results also indicated no yield benefit to fungicide application when the level of leaf diseases were low. Disease severity was slightly lower when fungicide was applied at flag leaf stage as opposed to anthesis, but both TW and TKW were slightly higher with the anthesis timing of fungicide. We conclude that a single fungicide application at anthesis, compared with flag leaf stage, will result in adequate control of leaf spotting diseases, equivalent yield, and improved grain quality. Anthesis fungicide application for leaf spot disease control is beneficial to wheat growers as this timing is optimum for control of fusarium head blight.

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