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Effect of Growth Stage on the Relationship Between Tan Spot and Spot Blotch Severity and Yield in Winter Wheat

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Effect of growth stage on the relationship between tan spot and spot blotch severity and yield in winter wheat

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Abstract
Foliar fungal diseases frequently cause significant economic losses in the hard red winter wheat production areas of the Great Plains of the United States. In 2007, field experiments were conducted in four environments in Nebraska, USA to determine the crop growth stage at which severity of tan spot and spot blotch was most strongly related to yield in winter wheat. Secondary objectives were to evaluate the efficacy of fungicides in controlling tan spot and spot blotch and to determine the effect of fungicide application timing on disease intensity and yield. Disease severity assessed at Zadoks growth stage (ZGS) 60 (flowering) had the strongest relationship to yield at all four locations ($0.72 \le R^2 \le 0.90$, $P < 0.0001$). Disease severity assessed at ZGS 71 (kernel watery ripe) also was strongly related to yield ($0.54 \le R^2 \le 0.87$, $P \le 0.0011$), but not as consistently across the four locations as disease severity assessed at ZGS 60. The relationship between yield and area under the disease progress curve (AUDPC) ($0.43 \le R^2 \le 0.80$, $P \le 0.0055$) was weaker and less consistent across the four locations than the relationship between yield and disease severity assessed at ZGS 60 or ZGS 71. Disease progress was faster at Mead (southeast) and Clay Center (south central) than at North Platte (west central) and Sidney (west). The fungicides azoxystrobin, pyraclostrobin, propiconazole, azoxystrobin plus propiconazole, and trifloxystrobin plus propiconazole effectively reduced disease severity and AUDPC. Out of a total of 60 fungicide treatments at four locations, 98%, 100%, and 100% significantly ($P = 0.05$) reduced disease severity, reduced AUDPC, and increased yield, respectively, compared to the check. Yield losses ranging from 27% to 42% were prevented by fungicide applications. There was no consistent effect on disease intensity or on yield of timing fungicide applications at ZGS 31 (first node on the stem detectable) versus ZGS 39 (ligule/collar of flag leaf just visible). The results from this study suggest that (i) the best predictor of yield loss caused by tan spot and spot blotch in winter wheat in Nebraska is disease severity assessed at flowering and (ii) fungicides can prevent significant yield losses from tan spot and spot blotch in winter wheat.

Keywords: tan spot, spot blotch, winter wheat, growth stage, fungicide application timing, disease severity, AUDPC, predictor, yield loss

1. Introduction

Hard red winter wheat (*Triticum aestivum* L.) is an economically important crop in the Great Plains of the United States. In 2007 in Nebraska, 2,294,000 t of winter wheat was harvested from 793,212 ha and valued at $522,536,000 (NASS, 2008). One of the major constraints in winter wheat production is the occurrence of yield-reducing diseases during the growing season. The most common foliar diseases of winter wheat in Nebraska are leaf rust (*Puccinia triticina*), tan spot (*Pyrenophora tritici-repentis*) (anamorph: *Drechslera tritici-repentis*), and spot blotch (*Cochliobolus sativus*) (anamorph: *Bipolaris sorokiniana*). Septoria tritici blotch (*Mycosphaerella graminicola*) (anamorph: *Septoria tritici*) occurs during excessively wet growing seasons. Stripe rust (*Puccinia striiformis* f. sp. *tritici*) and stem rust (*Puccinia graminis* f. sp. *graminis*) also occur, but less commonly. More recently (2007 and 2008), Fusarium head blight (*Gibberella zeae*) (anamorph: *Fusarium graminearum*) epidemics occurred in south central and eastern Nebraska due to excessively wet weather during the growing season.

Tan spot and spot blotch occur commonly in the Great Plains of the United States (Murray et al., 1998). The two diseases often are present together (Duveiller et al., 2005). Symptoms of tan spot include spots that appear initially as tan-brown flecks. The flecks expand into lens-shaped tan blotches with yellow borders. Large tan spot lesions coalesce and become darker at the centre due to formation of conidiophores and conidia of *D. tritici-repentis*. Spot blotch causes uniformly dark-brown, round to oblong lesions on leaves of wheat plants. *C. sativus* also causes common root rot and seedling blights in wheat (Murray et al., 1998; Wiese, 1987).

Dark, erumpent pseudothecia of *P. tritici-repentis* appear on wheat straw in autumn and are the means by which the fungus survives intercrop periods. *C. sativus* survives as a saprophyte in the soil and on previously parasitized host debris (Murray et al., 1998; Wiese, 1987). Because of the survival of both pathogens on host debris, incidence and severity of tan spot and spot blotch have increased over the last several decades because of a shift by growers towards conservation tillage practices that leave crop debris on the soil surface (Bailey, 1996; Bockus and Claassen, 1992; Duveiller et al., 2005). Both diseases are favoured by wet weather and temperatures above 20 °C and spores of the pathogens are spread by wind (Francl, 1997; Murray et al., 1998; Wiese, 1987).

Published research shows that yield losses caused by tan spot have been variable. In Australia, Rees et al. (1982) reported a yield loss of 49% in the most severely diseased treatment compared to a fungicide-sprayed treatment. In Oklahoma, Evans et al. (1999) found yield in plots inoculated with *P. tritici-repentis* to be 15% less than yield in fungicide-sprayed plots. Yield losses caused by spot blotch have similarly been variable. In Mexico, Villareal et al. (1995) found wheat yields in diseased plots to be 43% lower than yields in fungicide-treated plots. In growth chamber studies, De Milliano and Zadoks (1985) found a yield loss of 38% in African wheat cultivars. Yield losses of 40 and 85% were reported from the Philippines and Zambia, respectively (Lapis, 1985; Raemakers, 1988). Luz (1984) reported yield losses of 14 and 19% in two wheat cultivars in Brazil. In Nepal, yield losses of up to 43% were reported by Sharma and Duveiller (2006).

The majority of previous disease intensity–yield loss studies have focused on either tan spot (Evans et al., 1999; Rees and Platz, 1983; Rees et al., 1982; Shabeer and Bockus, 1988) or spot blotch (Luz, 1984; Sharma and Duveiller, 2006; Villareal et al., 1995), but not the two diseases together. Duveiller et al. (2005) reported an average yield loss of 30% from the tan spot/spot blotch disease complex in Nepal.

The relationship between disease intensity and yield can be used to estimate the expected yield loss during the growing season. This relationship has been shown to vary with the crop growth stage at which disease intensity is assessed. In Kansas, Shabeer and Bockus (1988) determined that 17% of the total yield loss in winter wheat from tan spot occurred due to early season infections by ascospores, and that 50% of the total yield loss had occurred by the boot stage. In Australia, Rees and Platz (1983) found that yield of wheat cv. Banks was reduced by 13% by early disease, 35% by late disease, and 48% by disease throughout the season. Research has not been done to determine the crop growth stage at which intensity of the tan spot/spot blotch disease complex is most strongly related to yield.

Tan spot and spot blotch, like other foliar fungal diseases of wheat, can be managed by various means including resistant cultivars, crop rotation, residue management, forecasting, biocontrol, and fungicide application (Bockus, 1998; Bockus et al., 2001; Bockus and Claassen, 1992; De Wolf and Francl, 1997; Evans et al., 1999; Sharma and Duveiller, 2003). With the widespread use of conservation tillage practices which leave crop residue on the soil surface, presently fungicide application is the most widely used management strategy in Nebraska.

Various studies have demonstrated the efficacy of fungicides in controlling foliar diseases of wheat and increasing yield. In Kansas, Kelley (2001) showed that over a period of 6 years, applications of the fungicide propiconazole significantly increased yield in winter wheat 77% of the time. Milus (1994) demonstrated efficacy of the fungicides tebuconazole, propiconazole, triadimefon, and mancozeb in controlling leaf rust and Septoria tritici blotch in Arkansas. Ransom and McMullen (2008) reported that the fungicides tebuconazole, tebuconazole plus prothioconazole, and pyraclostrobin were very effective in reducing leaf spots and Fusarium head blight in winter wheat in North Dakota. Comprehensive studies have not been done to evaluate the efficacy of fungicides in controlling foliar diseases of winter wheat under Nebraska conditions. Furthermore, in the last 10–15 years, fungicide chemistry has evolved considerably and now there are new fungicides on the market. There is a need to evaluate these fungicides for efficacy against foliar diseases of wheat.

The objectives of this study were to 1) determine the growth stage at which tan spot/spot blotch severity is most strongly related to yield in winter wheat, 2) evaluate the efficacy of fungicides in controlling tan spot and spot blotch in winter wheat under Nebraska conditions, and 3) determine the effect of fungicide application timing on tan spot and spot blotch intensity and yield in winter wheat.

2. Materials and methods

Seed of winter wheat cv. Millennium was planted with a small plot drill in autumn 2006 at the University of Nebraska's Agricultural Research and Development Center near Mead (26 Sep), the South Central Agricultural Laboratory near Clay Center (Sept. 27), the West Central Research and Extension Center near North Platte (Sept. 17), and the High Plains Agricultural Laboratory near Sidney (Sept. 13) (Figure 1). This cultivar was chosen because it has excellent resistance to leaf rust and stem rust (Baenziger et al., 2001). Hence, confounding effects from these diseases were minimized. Seeding rate was 72, 84, 72, and 50 kg ha^{-1} at Mead, Clay Center, North Platte, and Sidney, respectively. Standard agronomic practices for wheat production were followed at each location. Row spacing was 25.4 cm and plot size was 2.4 m × 2.4 m at Mead, Clay Center, and North Platte and 1.2 m by 6.7 m at Sidney.

Primary inoculum of *P. tritici-repentis* was provided naturally at all locations from pseudothecia on wheat straw from previous wheat crops. In Nebraska, wheat straw with pseudothecia accumulates in fields due to no-till or minimum tillage practices and inclusion of the wheat crop in rotation schemes throughout the state. Therefore, inoculum of *P. tritici-repentis* is readily available naturally. To ensure development of spot blotch, plots were inoculated with conidia of *B. sorokiniana* at Zadoks growth stage (ZGS) 30 (pseudostem erection) April 24, April 25, April 26, and April 27, at Sidney, North Platte, Clay Center, and Mead, respectively. Conidia were obtained by culturing mycelia from a single spore isolate of *B. sorokiniana* on V8 agar media in 9-cm-diameter Petri plates at 20 °C for 7–14 d in continuous darkness. Sterile distilled water was added to each Petri plate and conidia were dislodged with a rubber policeman. The conidial/mycelial suspension that resulted was filtered through several layers of cheesecloth to obtain the conidial suspension. Conidial concentration was determined with a haemacytometer. The isolate was obtained from wheat straw collected in 2006 from the University of Nebraska Agronomy Farm in Lincoln. Thirty millilitres of inoculum containing 70,000 conidia ml^{-1} m^{-2} were sprayed onto wheat leaves with a hand-pumped back pack sprayer. A second inoculation was similarly done at ZGS 31 (first node of stem detectable) on May 6, May 7, May 8, and May 9, at Sidney, North Platte, Clay Center, and Mead, respectively.

To generate different levels of disease intensity, the fungicides azoxystrobin + propiconazole (Quilt, Syngenta Crop Protection, Greensboro, NC), pyraclostrobin (Headline, BASF Ag Products, Research Triangle Park, NC), propiconazole (Tilt, Syngenta Crop Protection, Greensboro, NC), azoxystrobin (Quadris, Syngenta Crop Protection, Greensboro, NC) were each applied at a low rate and a high rate at ZGS 31, and at a high rate

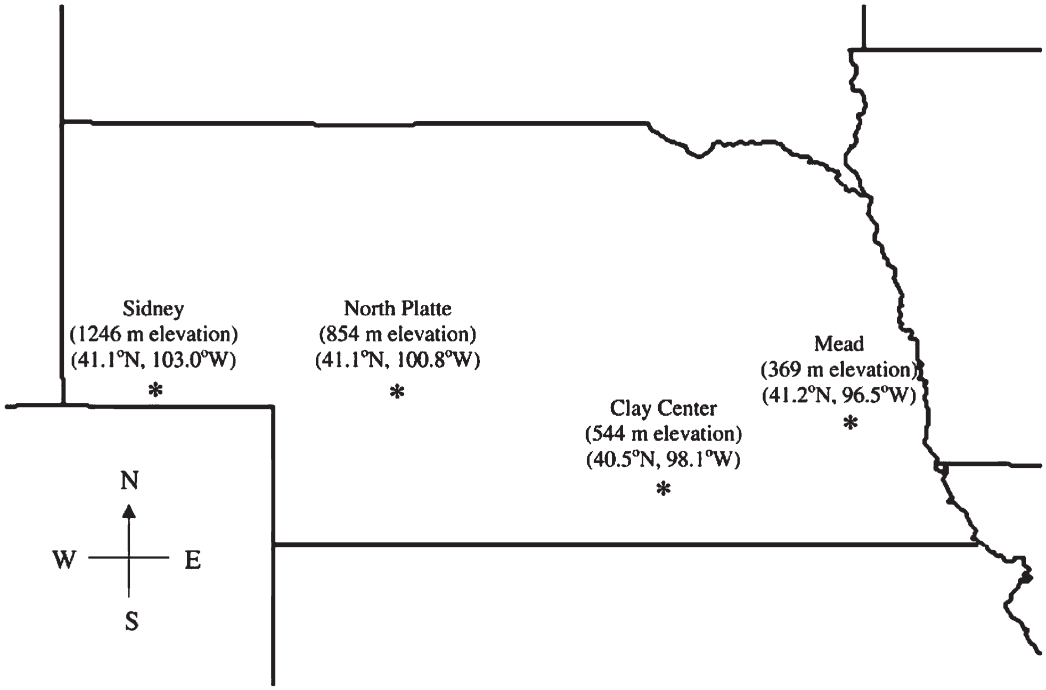

Figure 1. Map of Nebraska, USA (not to scale) showing the locations where field experiments were conducted in 2007 to determine the crop growth stage at which tan spot and spot blotch severity was most strongly related to yield and to evaluate the efficacy and application timing of fungicides in controlling tan spot and spot blotch in winter wheat cv. Millennium.

at ZGS 39 (ligule/collar of flag leaf just visible). Due to label restrictions, the fungicide trifloxystrobin + propiconazole (Stratego, Bayer CropScience, Research Triangle Park, NC) was applied at the same rate at ZGS 31, ZGS 31 and again at ZGS 39, and ZGS 39. Fungicides were applied with a CO_2-powered back pack sprayer set at 276 kPa, with a 1.2-m-wide boom and four Teejet # 800-1VS nozzles spaced 0.3 m apart. Treatments were arranged in randomized complete blocks with four replications.

Tan spot and spot blotch severity (%) was visually estimated together on the foliage of thirty plants at each of three arbitrarily selected sites per plot seven times during the growing season at ZGS 37 (flag leaf just visible), ZGS 39 (ligule/collar of flag leaf just visible), ZGS 55 (50% of inflorescence emerged), ZGS 60 (beginning of anthesis), ZGS 71 (kernel watery ripe), ZGS 75 (medium milk), and ZGS 85 (soft dough). Trapezoidal integration (Campbell and Madden, 1990; Madden et al., 2007; Shaner and Finney, 1977) was used to calculate area under the disease progress curve (AUDPC) from the seven disease severity assessments at each of the four locations. For each of the five fungicides used, disease progress curves were constructed for the treatments in which fungicide was applied at a full label rate, which is commonly used during commercial applications, at ZGS 31. ZGS 31 treatments were chosen to demonstrate disease progress from the earliest time fungicides were applied.

2.1. Data analysis

The GLM procedure of SAS (SAS Institute, Cary, NC) was used to analyze data. Fisher's protected least significant difference test at $P = 0.05$ (Gomez and Gomez, 1984; Steele et al., 1997) was used to compare pairs of treatment means. Linear regression analysis (Gomez and Gomez, 1984; Steele et al., 1997) was used to construct disease intensity–yield loss models. Treatment means were used in regression analysis. Coefficients of determination (R^2) and the t test and its associated P-value for the slope were used to determine the growth stage at which disease severity was most strongly related to yield.

3. Results

Disease development at all four locations was favoured by higher than normal rainfall and two inoculations with conidia of *B. sorokiniana*. During the months of May, June, and July, total rainfall for the 3 months was 27.9, 35.3, 38.5, and 20.8 cm at Mead, Clay Center, North Platte, and Sidney, respectively. Average temperature for the 3 months was 21.9, 21.6, 19.8, and 19.6 °C at Mead, Clay Center, North Platte, and Sidney, respectively.

The strongest relationship between disease severity and yield at all four locations was at flowering (ZGS 60) with $R^2 = 0.72$, $P < 0.0001$ (Mead), $R^2 = 0.89$, $P < 0.0001$ (Clay Center), $R^2 = 0.90$, $P < 0.0001$ (North Platte), and $R^2 = 0.86$, $P < 0.0001$ (Sidney) (Table 1). Disease severity at ZGS 71 also was strongly related to yield with $R^2 = 0.54$, $P = 0.0011$ (Mead), $R^2 = 0.80$, $P < 0.0001$ (Clay Center), $R^2 = 0.71$, $P < 0.0001$ (North Platte), and $R^2 = 0.87$, $P < 0.0001$ (Sidney). The relationship between AUDPC and yield was strongest at Clay Center ($R^2 = 0.80$, $P < 0.0001$), followed by North Platte ($R^2 = 0.66$, $P = 0.0001$), Sidney ($R^2 = 0.48$, $P = 0.0031$), and Mead ($R^2 = 0.43$, $P = 0.0055$) (Table 1).

In general, disease progression in the five fungicide treatments chosen for construction of disease progress curves was slow from the first to the fourth disease severity assessment date (first to third assessment date at Clay Center). Thereafter, disease severity increased exponentially up to the sixth assessment date (all treatments at Mead and four treatments at Clay Center) or the seventh assessment date (North Platte and Sidney and one treatment at Clay Center). This result was in contrast to the check treatment in which disease progression did not slow down initially except at North Platte (Figure 2). Disease progression during the entire growing season was slower at North Platte and Sidney compared to Mead and Clay Center. At Mead and Clay Center, 0.73 l ha^{-1} of trifloxystrobin + propiconazole applied at ZGS 31 and again at ZGS 39 slowed disease progression the most followed by 0.66 l ha^{-1} of pyraclostrobin applied at ZGS 31, whereas 1.02 l ha^{-1} of azoxystrobin + propiconazole applied at ZGS 31 slowed disease progression the least. At North Platte and Sidney, all five fungicide treatments slowed disease progression almost equally well (Figure 2C and D).

Differences in disease severity among treatments were highly significant ($P \leq 0.0003$) on all seven assessment dates (data not shown) at all four locations except the seventh assessment date at Mead ($P = 0.0183$), North Platte ($P = 0.0039$), and Sidney ($P = 0.0768$). Disease severity on the fourth assessment date is presented because it had the strongest relationship with yield at all four locations (Table 2). Differences in AUDPC among treatments were highly significant ($P < 0.0001$) at all four locations (Table 2).

At Mead, disease severity at flowering (fourth assessment date) ranged from 9% (0.66 l ha^{-1} of pyraclostrobin applied at ZGS 31) to 52% (check). AUDPC ranged from 1538% d (0.73 l ha^{-1} of trifloxystrobin + propiconazole applied at ZGS 31 and again at ZGS 39) to 2603% d (check). Yield ranged from 2854 kg ha^{-1} (check) to 4910 kg ha^{-1} (0.73 l ha^{-1} of trifloxystrobin + propiconazole applied at ZGS 31 and again at ZGS 39). There was a yield loss of 42% in the check treatment compared to the highest yielding treatment. Applying each fungicide at a lower rate at ZGS 31 or a higher rate at ZGS 31 or ZGS 39 did not result in significant differences in disease severity or yield at $P = 0.05$. For the same rate of each fungicide applied at ZGS 31 or ZGS 39, AUDPC was consistently lower ($P = 0.05$) in the ZGS 31 treatment. Applying trifloxystrobin + propiconazole at ZGS 31 and again at ZGS 39 significantly ($P = 0.05$) reduced disease severity and AUDPC compared to one application at ZGS 31 or ZGS 39 and resulted in a higher yield compared to one application at ZGS 31 (Table 2).

At Clay Center, disease severity at flowering ranged from 22% (0.73 l ha^{-1} of trifloxystrobin + propiconazole applied at ZGS 31 and again at ZGS 39) to 89% (check). AUDPC ranged from 1506% d (0.73 l ha^{-1} of trifloxystrobin + propiconazole applied at ZGS 31 and again at ZGS 39) to 3039% d (check). Yield was lowest in the check plots (3430 kg ha^{-1}) and highest in plots treated with 0.66 l ha^{-1} of pyraclostrobin at ZGS 39 (4713 kg ha^{-1}). Yield in the check treatment was 27% lower than yield in the highest yielding treatment. Applying the same rate of each fungicide consistently resulted in lower ($P = 0.05$) disease severity in the ZGS 39 than in the ZGS 31 treatment only for propiconazole and trifloxystrobin + propiconazole. There was no consistent effect of fungicide rate or application timing on AUDPC. For the same rate of each fungicide, yield was consistently higher ($P = 0.05$) in the ZGS 39 than in the ZGS 31 treatment for all fungicides except azoxystrobin. Applying trifloxystrobin + propiconazole at ZGS 31 and again at ZGS 39 significantly ($P = 0.05$) reduced disease severity and AUDPC and resulted in a higher ($P = 0.05$) yield compared to one application at ZGS 31 or ZGS 39 (Table 2).

At North Platte, disease severity at flowering was highest in check plots (28%) and lowest in plots treated with 0.88 l ha^{-1} of azoxystrobin at ZGS 39 (5%). AUDPC ranged from 1070% d (1.02 l ha^{-1} of azoxystrobin + propiconazole applied at ZGS 31) to 2014 % d (check). Yield was highest in plots treated with 0.66 l ha^{-1} of pyraclostrobin at ZGS 39 (4746 kg ha^{-1}) and lowest in the check plots (3131 kg ha^{-1}). Yield loss was 34% in the check plots compared to the highest yielding treatment. There was no consistent effect of fungicide rate or application timing on disease severity, AUDPC, or yield (Table 2).

At Sidney, disease severity at flowering ranged from 5.5% (0.73 l ha^{-1} of trifloxystrobin + propiconazole applied at ZGS 39) to 65% (check). AUDPC ranged from 963% d (1.02 l ha^{-1} of azoxystrobin + propiconazole applied at ZGS 31) to 2403% d (check). Yield ranged from 3738 kg ha^{-1} (check) to 5654 kg ha^{-1} (0.73 l ha^{-1} of trifloxystrobin + propiconazole applied at ZGS 31 and again at ZGS 39). Yield loss in check plots was 34% compared to the highest yielding treatment. Applying the same rate of each fungicide consistently resulted in a higher ($P = 0.05$) yield in the ZGS 39 than in the ZGS 31 treatment only for propiconazole (Table 2).

Out of 60 fungicide treatments at four locations, 98%, 100%, and 100% ($P = 0.05$) reduced disease severity, reduced AUDPC, and increased yield, respectively, compared to the check. Disease severity was negatively related to yield at all seven growth stages at all four locations except ZGS 37 and ZGS 39 at Clay Center. AUDPC was negatively related to yield at all four locations (Table 1).

Table 1. Coefficients of determination (R^2), intercepts, slopes, and the probability of a greater $|t|$ for the slope from regressions of yield (kg ha^{-1}) on disease severity (%) at various growth stages and on area under the disease progress curve (AUDPC, % d) in field experiments conducted to determine critical point models that best describe the relationship between yield and tan spot/spot blotch severity in wheat cv. Millennium at four locations in Nebraska, USA in 2007.

Location	Zadoks GS[a] AUDPC	Intercept	Slope	R^2	$P > \|t\|_{slope}$
Mead	37[b]	4583	−18.6	0.07	0.3287
	39	4439	−2.4	0.01	0.7918
	55	4543	−8.5	0.08	0.2891
	60	5266	−41.3	0.72	< 0.0001
	71	6235	−26.4	0.54	0.0011
	75	8097	−39.3	0.24	0.0530
	85	39424	−353.0	0.17	0.1150
	AUDPC	6709	−1.21	0.43	0.0055
Clay Center	37	4111	18.1	0.05	0.3957
	39	4282	1.2	0.00	0.8591
	55	4416	−5.5	0.05	0.3927
	60	5297	−19.2	0.89	< 0.0001
	71	5466	−16.8	0.80	< 0.0001
	75	5581	−14.4	0.45	0.0044
	85	17248	−130.1	0.21	0.0725
	AUDPC	6207	−0.9	0.80	< 0.0001
North Platte	37	4402	−17.9	0.04	0.4874
	39	4280	−0.8	0.00	0.9502
	55	4389	−7.7	0.02	0.5824
	60	5017	−64.7	0.90	< 0.0001
	71	5072	−32.8	0.71	< 0.0001
	75	5471	−20.4	0.49	0.0026
	85	8368	−42.9	0.26	0.0424
	AUDPC	6074	−1.4	0.66	0.0001
Sidney	37	5205	−1.2	0.00	0.9788
	39	5202	−0.4	0.00	0.9750
	55	5202	−0.3	0.00	0.9692
	60	5610	−28.6	0.86	< 0.0001
	71	6589	−36.2	0.87	< 0.0001
	75	6487	−27.3	0.52	0.0017
	85	7780	−28.4	0.11	0.2069
	AUDPC	5970	−0.6	0.48	0.0031

[a] Growth stage.
[b] Zadoks growth stages (ZGSs) at which disease severity assessments were made. ZGS 37, flag leaf just visible; ZGS 39, ligule/collar of flag leaf just visible; ZGS 55, half of inflorescence emerged; ZGS 60, beginning of anthesis; ZGS 71, kernel watery ripe; ZGS 75, medium milk; ZGS 85, soft dough.

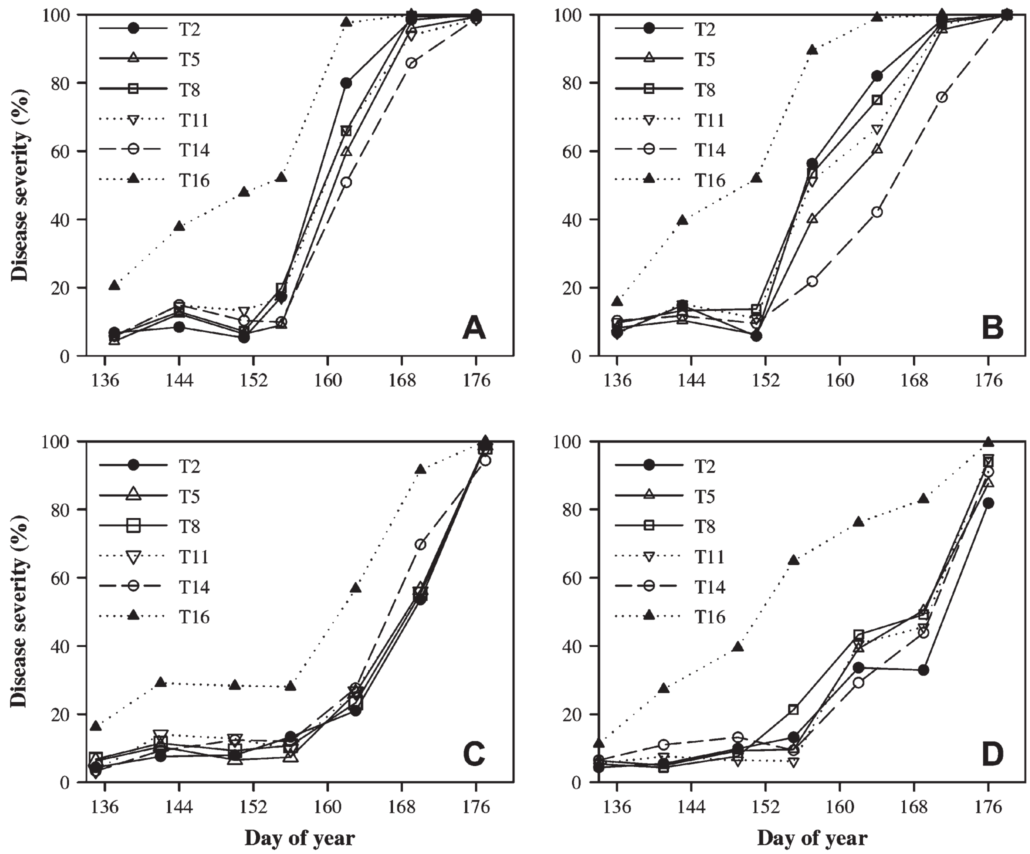

Figure 2. Disease progress curves in five fungicide treatments applied to winter wheat cv. Millennium at Zadoks growth stage (ZGS) 31 (first node on stem detectable) at Mead (A), Clay Center (B), North Platte (C), and Sidney (D), Nebraska, USA in 2007. T2, 1.02 l ha^{-1} of azoxystrobin + propiconazole; T5, 0.66 l ha^{-1} of pyraclostrobin; T8, 0.29 l ha^{-1} of propiconazole; T11, 0.88 l ha^{-1} of azoxystrobin; T14, 0.73 l ha^{-1} of trifloxystrobin + propiconazole applied at ZGS 31 and again at ZGS 39; T16, check. These were treatments in which fungicides were applied at full label rates, which are commonly used during commercial applications. ZGS 31 treatments were chosen to demonstrate disease progress from the earliest time fungicides were applied. Day of year: 136 (May 16); 144 (May 24); 152 (June 1); 160 (June 9); 168 (June 17); 176 (June 25).

4. Discussion

In this study, it was determined that out of seven disease severity assessments at different growth stages, tan spot/spot blotch severity assessed at flowering had the strongest relationship to yield. This result was consistent across four locations differing in climate and elevation. Few previous studies have determined the crop growth stage at which disease intensity is most strongly related to yield in wheat. Rees et al. (1982) found tan spot severity assessed from the late milk to the early dough growth stage to have a stronger relationship to grain yield, number of grains per head, and average grain weight compared to disease severity assessed at the medium milk growth stage. Results from our study differ from their results in that we found disease severity assessments made after ZGS 71 (kernel watery ripe) to have a weak relationship to yield (Table 2). Rees and Platz (1983) found that about 75% of the yield loss from tan spot occurred after jointing. They concluded that losses from tan spot were more likely to be greater in years when frequent rainfall occurred after jointing rather than before jointing. Shabeer and Bockus (1988) showed that the greatest yield loss from tan spot occurred when inoculation was done at the boot and the flowering growth stages, a result similar to that in our study. Disease progress curves indicated that disease progression was faster at Mead and Clay Center than at North Platte and Sidney. This observation can be attributed to a more favourable environment for disease development at Mead and Clay Center compared to North Platte and Sidney.

This study has demonstrated the efficacy of five fungicides in controlling tan spot and spot blotch in winter wheat. These fungicides are the most commonly used to control foliar diseases of wheat in Nebraska. Fungicide-treated plots consistently had lower ($P = 0.05$) disease severity and AUDPC than check plots at all four locations.

Yield losses ranging from 27% to 42% were recorded. Although only tan spot and spot blotch severity was assessed, other diseases were observed in research plots and likely contributed to these yield losses. The diseases observed were Septoria tritici blotch, leaf rust, powdery mildew, and barley yellow dwarf. Nevertheless, the yield losses observed in this study are similar to those reported in previous studies in other regions. In Nepal, Sharma and Duveiller (2006) reported grain yield losses from spot blotch of up to 38% in 2004 and 43% in 2005, respectively. In Brazil, Luz (1984) found that a natural epidemic of spot blotch reduced grain yields by 43%. Duveiller et al. (2005) reported an average yield reduction of 30% caused by the spot blotch/tan spot disease complex in Nepal. Under conditions favourable to disease development, Rees et al. (1982) measured a loss in grain yield of 49% from tan spot in Australia. In Oklahoma, check plots inoculated with *P. tritici-repentis* had 15% less yield than similarly inoculated fungicide-treated plots (Evans et al., 1999).

The effect of fungicide application timing on disease intensity and yield was variable. At Mead, applying a fungicide at ZGS 39 consistently resulted in higher ($P = 0.05$) AUDPC than applying the same rate of the fungicide at ZGS 31 for all five fungicides. At Mead and Clay Center, two applications of trifloxystrobin + propiconazole (at ZGS 31 and again at ZGS 39) significantly ($P = 0.05$) reduced disease severity and AUDPC and increased yield compared to one application at ZGS 31 or ZGS 39. This result was expected as the two applications prolonged the period of disease control compared to one application.

Applying foliar fungicides earlier than ZGS 39 may be warranted and may be beneficial if environmental conditions favour development of severe disease early in the growing season. Disease developed earlier at Mead than at Clay Center (Figure 2 T16, check), and this may be the reason why a ZGS 31

Table 2. Combined tan spot and spot blotch severity and AUDPC, and yield from experiments conducted to determine the crop growth stage at which tan spot/spot blotch severity is most strongly related to yield and to evaluate the efficacy and application timing of fungicides in winter wheat cv. Millennium at four locations in Nebraska, USA in 2007.

Location[a]	Mead			Clay Center			North Platte			Sidney		
Fungicide treatment[b]	Severity (%)	AUDPC (% d)	Yield (kg ha^{-1})	Severity (%)	AUDPC (% d)	Yield (kg ha^{-1})	Severity (%)	AUDPC (% d)	Yield (kg ha^{-1})	Severity (%)	AUDPC (% d)	Yield (kg ha^{-1})
Azoxystrobin + propiconazole ZGS[d] 31, 0.51 l ha^{-1}	21.4 bc[c]	1867 c–e	4464 ab	57.9 b–e	2197 b–d	4195 e–g	20.5 ab	1273 bc	3895 c	18.5 bc	1156 d–f	5072 c
Azoxystrobin + propiconazole ZGS 31, 1.02 l ha^{-1}	17.3 b–d	1804 e	4267 b	56.3 b–e	2155 b–e	4054 g	13.3 b–d	1070 c	4320 a–c	13.2 b–d	963 f	5003 c
Azoxystrobin + propiconazole ZGS 39, 1.02 l ha^{-1}	23.2 bc	2132 b	4675 ab	46.8 e–i	2179 b–d	4435 cd	8.0 de	1284 bc	4737 a	8.7 cd	1302 b–d	5377 a–c
Pyraclostrobin ZGS 31, 0.44 l ha^{-1}	15.6 cd	1774 e	4357 ab	61.8 bc	2175 b–d	4162 fg	9.0 de	1234 bc	4498 a–c	8.4 cd	1205 c–e	5158 bc
Pyraclostrobin ZGS 31, 0.66 l ha^{-1}	9.0 d	1621 fg	4583 ab	40.0 g–i	1852 fg	4381 cd	7.4 de	1112 c	4473 a–c	9.7 cd	1122 d–f	5195 a–c
Pyraclostrobin ZGS 39, 0.66 l ha^{-1}	19.3 bc	1895 c–e	4648 ab	36.0 hi	1775 g	4713 a	5.2 de	1099 c	4746 a	6.3 d	1210 c–e	5385 a–c
Propiconazole ZGS 31, 0.15 l ha^{-1}	20.8 bc	1854 de	4222 b	53.3 c–e	2173 b–e	4433 cd	8.6 c–e	1158 c	4406 a–c	12.9 b–d	1108 d–f	5011 c
Propiconazole ZGS 31, 0.29 l ha^{-1}	19.9 bc	1765 ef	4473 ab	53.5 c–f	2135 b–e	4295 d–f	10.8 c–e	1129 c	4278 a–c	21.3 b	1220 c–e	5024 c
Propiconazole ZGS 39, 0.29 l ha^{-1}	23.5 bc	2132 b	4764 ab	34.4 ij	1966 e–g	4711 a	7.8 de	1125 c	4519 ab	15.3 b–d	1468 b	5560 ab
Azoxystrobin ZGS 31, 0.29 l ha^{-1}	22.7 bc	1952 cd	4455 ab	60.7 b–d	2288 b	4140 fg	16.5 bc	1186 c	3901 c	12.4 b–d	1171 d–f	5303 a–c
Azoxystrobin ZGS 31, 0.88 l ha^{-1}	17.0 b–d	1758 ef	4403 ab	51.3 c–g	2045 c–f	4420 cd	9.8 c–e	1180 c	4217 a–c	6.2 d	1098 d–f	5442 a–c
Azoxystrobin ZGS 39, 0.88 l ha^{-1}	24.0 bc	2011 bc	4212 b	41.3 f–i	1995 d–f	4527 bc	4.9 e	1119 c	4651 a	10.5 b–d	1410 bc	5483 a–c
Trifloxystrobin + propiconazole ZGS 31, 0.73 l ha^{-1}	24.8 b	1904 c–e	4201 b	68.7 b	2290 b	4052 g	10.9 c–d	1296 bc	4242 a–c	8.2 cd	1229 c–e	5283 a–c
Trifloxystrobin + propiconazole ZGS 31, (0.73 l ha^{-1}) and ZGS 39 (0.73 l ha^{-1})	9.9 d	1538 g	4910 a	21.8 j	1506 h	4685 ab	12.0 c–e	1245 bc	4321 a–c	9.4 cd	1087 ef	5449 a–c
Trifloxystrobin + propiconazole ZGS 39, 0.73 l ha^{-1}	21.9 bc	2107 b	4637 ab	48.7 d–h	2223 bc	4331 de	12.9 c–e	1460 b	3931 bc	5.5 d	1389 bc	5654 a
Check	52.1 a	2603 a	2854 c	89.4 a	3039 a	3430 h	28.0 a	2014 a	3131 d	64.9 a	2403 a	3738 d

[a] Mead, Clay Center, North Platte, and Sidney are located, respectively, in southeastern, south central, west central, and western Nebraska.
[b] To generate different levels of disease severity, five fungicides commonly used to control foliar diseases of wheat were each applied in three treatments at rates within the range specified on the label. The treatments were: apply at Zadoks growth stage 31 (ZGS 31, first node detectable on the stem) at a low rate; apply at ZGS 31 at a high rate; and apply at ZGS 39 (ligule/collar of flag leaf just visible) at a high rate. Due to label restrictions, the fungicide trifloxystrobin + propiconazole was applied at the same rate at ZGS 31, ZGS 31 and again at ZGS 39, and ZGS 39.
[c] Means followed by the same letter within a column are not significantly different according to Fisher's protected least significant difference test at $P = 0.05$.
[d] Zadoks growth stage.

application at Mead controlled disease better than a ZGS 39 application. Previous studies have demonstrated yield loss from early season infections and a benefit from early fungicide applications. Shabeer and Bockus (1988) found that about 17% of total yield loss from tan spot occurred from early season infections, which strengthens the need for pre-ZGS 39 applications under conditions favourable to disease development. Marroni et al. (2006) found that the lowest AUDPC and the best level of protection against early season Septoria tritici blotch were achieved with azoxystrobin applied at the pre-stem extension stage of crop growth. They also found good control of the disease when a mixture of azoxystrobin and epoxiconazole was applied at the pre-stem extension stage or at the stem extension stage.

Our results are similar to previous studies which also found inconsistency in the effects of fungicide application timing for control of foliar diseases of wheat. Cromey et al. (2004) found no consistent effects of crop growth stage when the fungicides azoxystrobin and tebuconazole were applied at three alternative growth stages between flag leaf emergence and flowering to control *Didymella exitialis* (anamorph: *Ascochyta* spp.). Bockus et al. (1997) found the optimum timing to be between the boot and the fully headed growth stages. Duczek and Jones-Flory (1994) found the optimum timing to be between extension of the flag leaf and the medium milk growth stages. Wiersma and Motteberg (2005) found that across cultivars, the optimum timing for foliar fungicide application was ZGS 60 rather than ZGS 39. The results from our study suggest that applying a fungicide at ZGS 31 and again at ZGS 39 may reduce disease intensity and increase yield more than one application at ZGS 31 or ZGS 39. However, applications at ZGS 31 and again at ZGS 39 may not be cost-effective depending on disease levels, fungicide cost, application cost, and the price of wheat.

This study has demonstrated that tan spot and spot blotch severity assessed at flowering was consistently (across four locations) most strongly related to yield. It is concluded that tan spot and spot blotch severity assessed at flowering is a better predictor of yield loss than AUDPC or disease severity assessed at other crop growth stages. Disease progressed faster at Mead and Clay Center than at North Platte and Sidney. This difference was attributed to a climate more favourable to disease development at Mead and Clay Center compared to North Platte and Sidney. The efficacy of five commonly used foliar fungicides in controlling tan spot and spot blotch was also demonstrated. Fungicide applications prevented yield losses ranging from 27% to 42%. There was no consistent effect of fungicide application timing on disease severity, AUDPC, or yield. Although fungicides are routinely timed to protect the flag leaf in Nebraska, the decision to apply a fungicide usually depends on local environmental conditions and when disease is detected.

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