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### Yield Response to Foliar Fungicide Application in Winter Wheat

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#### 1. Introduction

Fungicides are routinely applied to control fungal diseases of wheat and other cereal crops, with the main goal of preventing yield loss (or increasing yield) and hence maximizing economic returns. In North America, the fungicides used to control foliar fungal diseases of wheat belong to two major classes with a broad spectrum of activity against fungal pathogens. These are the strobilurins and triazoles. Fungicides in both classes are used as foliar fungicides and seed treatments. The strobilurins are named in recognition of a mushroom, *Strobilurus tenacellus*, the original source of the chemical compound that formed the basis of the chemistry of this fungicide class. They are quinone outside inhibitors (QoI) and work by interfering with energy production in fungi (Vincelli, 2002). They act as local systemics by inhibiting fungal spore germination and early infection, and are highly effective when applied preventively. The strobilurins have a single-site mode of action. Examples of strobilurin fungicides used in cereal crop production in North America are azoxystrobin, pyraclostrobin and trifloxystrobin.

The triazoles are characterized by having a five-membered ring of two carbon atoms and three nitrogen atoms. They are curative and move systemically through the plant xylem. Triazoles slow fungal growth through the inhibition of sterol biosynthesis (Buchenauer, 1987). Sterols are essential building blocks of fungal cell membranes and are inhibited at a single site by triazoles. Because of their curative activity against early fungal infections and their ability to redistribute in the crop, triazoles are highly effective and reliable (Hewitt, 1998). Examples of triazoles used in cereal crop production in North America are metconazole, propiconazole, prothioconazole, and tebuconazole.

In the Great Plains of the United States, the most common foliar diseases of winter wheat are leaf rust (*Puccinia triticina*), powdery mildew (*Blumeria graminis* f. sp. graminis), tan spot (*Pyrenophora tritici-repentis*) (anamorph: *Drechslera tritici-repentis*), Septoria tritici blotch (*Mycosphaerella graminicola*) (anamorph: *Septoria tritici*), spot blotch (*Cochliobolus sativus*) (anamorph: *Bipolaris sorokiniana*), and Stagonospora nodorum blotch (*Phaeosphaeria nodorum*) (anamorph: *Stagonospora nodorum*). Stripe rust (*Puccinia striiformis* f. sp. *tritici*) and stem rust (*Puccinia graminis* f. sp. *tritici*) also occur, but less commonly.

The magnitude of yield loss caused by these diseases in winter wheat is variable and depends on several factors including environmental conditions during the growing season, cultural practices, and cultivar resistance. Leaf rust occurs every year in the wheat-producing regions of the U.S. In 2007, severe epidemics of leaf rust occurred in the Great Plains region of North America, causing yield losses of up to 14% (Kolmer et al., 2009). Stripe rust is more frequent in the western U.S., especially the Pacific Northwest (Sharma-Poudyal & Chen, 2011). However, it can be widespread in certain years, as in 2010 when severe epidemics occurred throughout the wheat-producing regions of North America. Yield losses of up to 74% due to stripe rust have been documented in experimental fields (Sharma-Poudyal & Chen, 2011). Stem rust has been effectively controlled in the U.S. through genetic resistance and eradication of barberries (*Berberis vulgaris* and *B. Canadensis*), which act as alternate hosts. Stem rust has the potential to cause 100% yield loss (Murray et al., 1998). Powdery mildew occurs wherever wheat is grown and is common where high humidity prevails during the growing season. Yield losses of up to 25% due to powdery mildew have been reported (Murray et al., 1998).

Spot blotch occurs commonly in the Great Plains of the United States (Murray et al. 1998). The causal agent, *C. sativus*, also causes common root rot and seedling blights in wheat. Spot blotch often occurs together with tan spot (Duveiller et al., 2005). In wet growing seasons, Septoria tritici blotch also can occur as part of this foliar disease complex. This leaf spot disease complex is favored by cultural practices that leave crop residue on the soil surface (Watkins & Boosalis, 1994). Yield losses of up to 50% have been documented to be caused by these leaf spot diseases in winter wheat (Murray et al. 1998; Villareal et al., 1995; Wegulo et al., 2009).

### 2. The use of fungicides to control foliar fungal diseases of wheat

Fungicides have been used routinely in cereal production since the development of systemics in the late 1960s (Hewitt, 1998). New fungicide chemistries have been developed steadily over the last several decades, in part to increase efficacy and overcome resistance to older chemistries in pathogen populations. The benefits of fungicide use in crop production have long been acknowledged. Ordish and Dufour (1969) noted the popularity of spraying fungicides to control crop diseases; returns of up to three times the cost involved often were realized from fungicide application. In the United Kingdom, experiments conducted from 1978 to 1982 showed that applying fungicides to winter wheat resulted in a yield response of up to 89%, and the value of the increased yield from fungicide application to cereals in 1982 was nearly double the fungicide costs (Cook and King, 1984). In Denmark, fungicide application to control powdery mildew and *Septoria* diseases resulted in yield increases of 400-2700 kg ha<sup>-1</sup> with margin over cost varying from -500 kg ha<sup>-1</sup> to 2000 kg ha<sup>-1</sup> (Jørgensen et al., 2000). An economic evaluation of fungicide use in winter wheat in Sweden showed a mean net return of US\$28 ha<sup>-1</sup> during the period 1995-2007 and \$16 ha<sup>-1</sup>during the period 1983-2007 (Wiik and Rosenqvist, 2010).

In the U.S., various studies have demonstrated yield increases in winter wheat due to fungicide application. Wegulo et al. (2009) showed that up to 42% yield loss was prevented by applying foliar fungicides to winter wheat. Kelley (2001) found that over a period of six years, the fungicide propiconazole significantly increased winter wheat yield 77% of the time. Vamshidhar et al. (1998) demonstrated significant yield increases from fungicide application to control the disease complex of leaf rust, tan spot, and Septoria tritici blotch in

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winter wheat. They found that cultivar specific economic benefits were associated with improved wheat quality from fungicide treatment. Ransom and McMullen (2008) showed that within an environment and averaged across winter wheat cultivars, fungicides improved yields by 5.5 to 44.0%. Tebuconazole applied at Zadoks growth stage (GS) 37 (Zadoks, 1974) and propiconazole applied at GS 37 followed by triadimefon + mancozeb at GS 55 to control leaf rust and Septoria tritici blotch consistently resulted in the lowest disease severities and highest winter wheat yields (Milus, 1994).

In the Great Plains region of the U.S., the prevalence, incidence, and severity of tan spot and other residue-borne diseases such as spot blotch and Septoria tritici blotch have increased over the last several decades due to a shift toward conservation tillage practices that leave crop debris on the soil surface (Watkins and Boosalis, 1994). The damage caused by these and other foliar fungal diseases has promoted the use fungicides in winter wheat production in the region.

#### 3. Timing of foliar fungicide application in winter wheat

Fungicides are generally applied to winter wheat 1-2 times per season. Some farmers apply a fungicide early in the growing season during the stem elongation growth stage to control early season diseases such as tan spot. Often these early fungicide applications are done in combination with herbicide or fertilizer application. A second fungicide application is usually timed to protect the flag leaf. A high risk of Fusarium head blight may necessitate a third fungicide application at early flowering. Results from previous studies on the effect of fungicide application timing on yield in winter wheat have been inconsistent. Some studies have demonstrated yield loss from early season infections and a benefit from early fungicide application in winter wheat. Shabeer and Bockus (1988) found that about 17% of total yield loss from tan spot occurred from early season infections. Marroni et al. (2006) found that the lowest area under the disease progress curve (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. 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 GS 60 rather than GS 39. Because of the inconsistent results from previous studies, experiments were conducted in Nebraska, USA to investigate the effects of fungicides and fungicide application timing on disease severity, yield and economic returns in winter wheat.

#### 4. Methods

#### 4.1 Field experiments

The methods used in field experiments have been described previously (Wegulo et al., 2009; Wegulo et al., 2011).

#### 4.1.1 2006 field experiments

In autumn 2005, seed of winter wheat cv. Millennium was planted with a small plot drill at the University of Nebraska's Agricultural Research and Development Center (ARDC) near Mead (9 Oct), the South Central Agricultural Laboratory (SCAL) near Clay Center (22 Sep), the West Central Research and Extension Center (WCREC) near North Platte (21 Sep), and the High Plains Agricultural Laboratory (HPAL) near Sidney (6 Sep) (Fig. 1).



Fig. 1. Map of Nebraka, USA (not to scale) showing the locations where field experiments were conducted in 2006 and 2007 to determine the effects of fungicides and fungicide application timing on foliar fungal disease severity, yield increase and net return in winter wheat cv. Millennium.

Standard agronomic practices for wheat production were followed at each location. Seeding rate was 98, 84, 72, and 50 kg ha<sup>-1</sup> at Mead, Clay Center, North Platte, and Sidney, respectively. Row spacing was 25.4 cm and plot size was 1.8 m x 4.6 m at Mead, 1.2 m x 8.2 m at Clay Center and Sidney, and 2.1 m x 4.6 m at North Platte. Four fungicides were each applied once at GS 31 (first node on the stem detectable) or GS 37 (flag leaf just visible) (Table 1). The fungicides were azoxystrobin (7.0% of marketed product) + propiconazole (11.7%) (Quilt, Syngenta Crop Protection, Greensboro, NC), pyraclostrobin (23.6%) (Headline, BASF Ag Products, Research Triangle Park, NC), azoxystrobin (22.9%) (Quadris, Syngenta Crop Protection, Greensboro, NC), and trifloxystrobin (11.4%) + propiconazole (11.4%) (Stratego, Bayer CropScience, Research Triangle Park, NC). Treatments were arranged in randomized complete blocks with four replications.

Due to minimum or no-tillage practices and inclusion of winter wheat in crop rotation schemes, primary inoculum of *P. tritici-repentis* was provided naturally at all locations from pseudothecia on wheat straw from previous wheat crops. Inoculum of other fungal foliar pathogens such as *M. graminicola*, *B. graminis* f.sp. *tritici*, and *P. triticina* also occurred naturally. At GS 31, plots were inoculated with conidia of *B. sorokiniana* on 2 May at Mead and Clay Center and on 5 May at Sidney and North Platte. 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 to 14 days 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 cheese cloth to obtain the conidial suspension. Conidial concentration was determined with a haemacytometer.

Thirty millilitres of inoculum containing 70 000 conidia ml<sup>-1</sup> m<sup>-2</sup> were sprayed onto wheat leaves in each plot with a hand-pumped back pack sprayer. Fungicide treatments were applied 24 h after inoculation at each location. Fungicides were applied with a CO<sub>2</sub>-powered back pack sprayer set at 276 kPa, with a 1.2-m-wide boom and 4 Teejet # 800-1VS nozzles spaced 0.3 m apart. Tan spot and spot blotch severity (%) was visually estimated together on the flag leaf of thirty randomly selected plants per plot at growth stage GS 55 (50% of inflorescence emerged) at Sidney and GS 60 (beginning of anthesis) at Mead, Clay Center, and North Platte. At maturity, plots were harvested with a small plot combine and grain yield was determined.

#### 4.1.2 2007 field experiments

Seed of winter wheat cv. Millennium was planted with a small plot drill in autumn 2006 at the University of Nebrasaka's Agricultural Research and Development Center near Mead (26 Sep), the South Central Agricultural Laboratory near Clay Center (27 Sep), the West Central Research and Extension Center near North Platte (17 Sep), and the High Plains Agricultural Laboratory near Sidney (13 Sep) (Fig. 1). Standard agronomic practices for wheat production were followed at each location. Seeding rate was 72, 84, 72, and 50 kg ha<sup>-1</sup> at Mead, Clay Center, North Platte, and Sidney, respectively. Row spacing was 25.4 cm and plot size was 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. To ensure development of spot blotch, plots were inoculated with conidia of *B. sorokiniana* at GS 30 (pseudostem erection) on 24 Apr, 25 Apr, 26 Apr, and 27 Apr at Sidney, North Platte, Clay Center, and Mead, respectively. A second inoculation was similarly done at GS 31 (first node of stem detectable) on 6 May, 7 May, 8 May, and 9 May at Sidney, North Platte, Clay Center, and Mead, respectively. Inoculum was obtained, prepared, and applied as in 2006.

Five fungicides were each applied once at GS 31 (first node on the stem detectable) or GS 39 (ligule/collar of flag leaf just visible) (Table 1). The fungicides were azoxystrobin (7.0% of marketed product) + propiconazole (11.7%) (Quilt, Syngenta Crop Protection, Greensboro, NC), pyraclostrobin (23.6%) (Headline, BASF Ag Products, Research Triangle Park, NC), propiconazole (41.8%) (Tilt, Syngenta Crop Protection, Greensboro, NC), azoxystrobin (22.9%) (Quadris, Syngenta Crop Protection, Greensboro, NC), and trifloxystrobin (11.4%) + propiconazole (11.4%) (Stratego, Bayer CropScience, Research Triangle Park, NC). Fungicides were applied with a CO<sub>2</sub>-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 at GS 60 (beginning of anthesis). At maturity, plots were harvested with a small plot combine and grain yield was determined.

#### 4.2 Economic analysis

Table 1 shows the fungicide costs, fungicide application cost, and wheat prices used in economic analysis. Average wheat prices were calculated from data provided by the USDA Agricultural Marketing Service. The average local prices during thirteen months were used. The months used were August prior to crop planting to August following crop harvest in 2005-2006 and 2006-2007. Fungicide prices (\$ ha<sup>-1</sup>) were obtained by surveying local retailers and chemical manufacturers and averaged. Adjuvant and surfactant costs were omitted because of the wide variation in their uses and costs. Fungicide application costs were obtained by surveying commercial applicators in Nebraska. All surveys were conducted in 2009 by telephone. Information provided by those surveyed was obtained from 2006 and 2007 records. Aerial application cost was used. Because aerial fungicide application is by contract between the grower and the commercial applicator, machinery and machinery maintenance costs were omitted.

2006			2007		
Fungicide treatment	Fungicide Cost (\$ ha <sup>-1</sup> )	Fungicide application cost (\$ ha <sup>-1</sup> )	Fungicide treatment	Fungicide cost (\$ ha <sup>-1</sup> )	Fungicide Application cost (\$ ha <sup>-1</sup> )
Quilt GS 31, 0.58 l ha <sup>-1</sup>	16.83	18.19	Quilt GS 31, 1.02 l ha <sup>-1</sup>	32.76	18.19
Quilt GS 37, 0.58 l ha-1	16.83	18.19	Quilt GS 39, 1.02 l ha-1	32.76	18.19
Headline GS 31, 0.58 l ha <sup>-1</sup>	27.48	18.19	Headline GS 31, 0.66 l ha <sup>-1</sup>	34.22	18.19
Headline GS 37, 0.58 l ha-1	27.48	18.19	Headline GS 39, 0.66 l ha <sup>-1</sup>	34.22	18.19
Quadris GS 31, 0.58 l ha <sup>-1</sup>	35.21	18.19	Tilt GS 31, 0.29 l ha-1	27.08	18.19
Quadris GS 37, 0.58 l ha <sup>-1</sup>	35.21	18.19	Tilt GS 39, 0.29 l ha <sup>-1</sup>	27.08	18.19
Stratego GS 31, 0.73 l ha-1	25.38	18.19	Quadris GS 31, 0.88 l ha <sup>-1</sup>	58.73	18.19
Stratego GS 37, 0.73 l ha <sup>-1</sup>	25.38	18.19	Quadris GS 39, 0.88 l ha <sup>-1</sup>	58.73	18.19
			Stratego GS 31, 0.73 l ha <sup>-1</sup>	28.17	18.19
			Stratego GS 39, 0.73 l ha-1	28.17	18.19

Table 1. Fungicide treatments and fungicide and fungicide application costs used to calculate net return from applying fungicides to winter wheat cv. Millennium to control foliar fungal diseases at four locations in Nebraska, USA in 2006 and 2007.

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Net return from fungicide application was calculated as

$$R_n = Y_i P - (F_c + A_c) \tag{1}$$

where  $R_n$  is the net return from fungicide application (\$ ha<sup>-1</sup>);  $Y_i$  is yield increase from fungicide application (kg ha<sup>-1</sup>), obtained by subtracting the yield in the check treatment from the yield in the fungicide treatments; *P* is the wheat price (\$ kg<sup>-1</sup>); *F<sub>c</sub>* is the fungicide cost (\$ ha<sup>-1</sup>); and  $A_c$  is the fungicide application cost (\$ ha<sup>-1</sup>).

#### 4.3 Data analysis

Data from each of the four locations were subjected to analysis of variance using the the GLM procedure of SAS (SAS Institute, Cary, NC). These data from individual locations have been published previously (Wegulo et al., 2009; Wegulo et al., 2011). To determine the overall effect of fungicides and fungicide application timing on disease severity, yield increase, and net return (the data reported in this chapter), the means from each location were further subjected to analysis of variance using the GLM procedure of SAS. In this latter analysis, each of the four locations was considered a replication. Fisher's least significant difference test at P = 0.05 (Gomez and Gomez, 1984) was used to compare pairs of treatment means. Linear regression analysis (Gomez and Gomez, 1984) was used to model the relationships between disease severity and yield increase, between disease severity and net return, and between yield increase and net return with disease severity and yield increase as independent variables and yield increase and net return as dependent variables.

#### 4.4 Results and discussion

#### 4.4.1 Effect of weather on disease severity

Average total rainfall across the four locations for the months of May, June, and July (the period of active vegetative growth and grain filling in the winter wheat crop in Nebraska) was 15.6 cm in 2006 and 30.6 cm in 2007 (Table 2). Therefore, although average temperature was similar in both years, the growing season in 2006 was unusually dry whereas it was excessively wet in 2007. Consequently, disease severity was very low in 2006 compared to 2007 (Table 2).

Environment has a major influence on the development of plant disease epidemics (Campbell and Madden, 1990). Temperature and moisture are especially critical to the development, reproduction, and survival of plant pathogens. In this study, since temperature was similar in both years, the difference in disease severity between 2006 and 2007 was attributable to moisture. In 2006, dry conditions considerably slowed down disease development, resulting in very low disease severity. Excessive moisture in 2007 favored the development of severe epidemics, resulting in considerable disease severity even in sprayed plots (Tables 2 and 3).

This study demonstrates that variation in weather from year to year can significantly impact not only disease development but overall yield. Average yield in sprayed plots in 2006 was 36.4% less than average yield in sprayed plots in 2007 (Table 2). This was likely because of lack of adequate moisture during the grain filling period in 2006. Due to the low disease severity in 2006, the average yield from unsprayed plots was only 12.6% lower than the average yield in sprayed plots compared to 2007 when the average yield from unsprayed plots was 29.4% lower than the average yield from sprayed plots.

This variable effect of weather on disease and yield has been demonstrated in other studies. Using results from fungicide field trials conducted from 1983 to 2007 and disease surveys conducted from 1988 to 2007 in winter wheat in southern Sweden, Wiik and Elwadz (2009) showed through regression analysis that air temperature and precipitation explained more than 50% of the variation in yield increase between years. They found May precipitation to be the factor most consistently related to Septoria tritici blotch, Stagonospora nodorum blotch, and tan spot. In the UK, Gladders et al. (2001) showed that year to year variation in the severity of Septoria tritici blotch was greater than spatial variation.

	2006	2007
Average total rainfall (cm)	15.6	30.6
Average temperature (°C)	21.3	20.7
Average disease severity (%)		
Sprayed plots	2.4	21.8
Unsprayed plots	4.3	58.6
Average yield (kg ha-1)		
Sprayed plots	2963	4658
Unsprayed plots	2589	3288
Average yield increase (kg ha-1)	394	1369
Average net return (\$ ha-1)	12	189
Probability of a positive net return	0.63	1.00

Table 2. Average total rainfall and temperature (May, June, and July), disease severity, yield increase, and net return; and the probability of a positive net return from experiments conducted to determine the effects of fungicides and fungicide application timing on disease severity, yield increase and net return in winter wheat cv. Millennium in Nebraska, USA in 2006 and 2007.

#### 4.4.2 Effects of fungicides and fungicide application timing on disease severity

There were no significant differences among fungicides in their efficacy in controlling disease in both application timings in both years (Table 3). In 2006, disease severity in unsprayed plots (4.3%) was significantly higher than that in plots sprayed with all fungicides except Stratego in the GS 31 application timing. In 2007, disease severity in unsprayed plots was significantly higher than in all sprayed plots. Although fungicides did not significantly differ in the level of disease control in each year, Headline was the most efficacious, especially in 2007 when disease severity was high. Disease severity did not significantly differ between the two fungicide application timings for any of the fungicides (Table 3) or when averaged across fungicides (Fig. 2), but generally was higher in the GS 31 than in the GS 37/GS 39 application timing. This was expected since the time between fungicide application and disease assessment was longer in the earlier (GS 31) application timing. This resulted in a greater reduction in fungicide residual activity in the earlier application timing, leading to higher disease severity in this timing compared to the later (GS 37/GS 39) timing.

In a previous study in the UK, Cook et al. (1999) showed that the effect of fungicide application timing on disease intensity varied with the fungicide applied and the disease

controlled. Reduction in Septoria tritici blotch (STB) area under the disease progress curve (AUDPC) was greater when chlorothalonil was applied at GS 35-39 than at GS <35. Propiconazole reduced STB AUDPC only slightly, reduced powdery mildew and leaf rust AUDPC significantly, and reduced stripe rust AUDPC equally well when applied at GS 35-39 compared to GS <35. In general, efficacy of fungicides in controlling disease declined as the growth stage at which the fungicides were applied increased (Cook et al., 1999). In our study, fungicides were not applied beyond GS 39 and only leaf spot disease severity was assessed.

2006			2007		
Fungicide	Disease	Disease		Disease	Disease
treatment	GS 31	GS 37	Fungicide treatment	GS 31	GS 39
	timing	timing		timing	timing
Headline 0.58 l ha-1	2.0 b	2.2 b	Headline 0.66 l ha-1	16.5 b	16.7 b
Quadris 0.58 l ha-1	2.4 b	2.0 b	Quadris 0.88 l ha-1	21.1 b	20.2 b
Quilt 0.58 l ha <sup>-1</sup>	2.7 b	2.5 b	Quilt 1.02 l ha-1	25.0 b	21.7 b
Stratego 0.73 l ha-1	3.2 ab	2.2 b	Stratego 0.73 l ha-1	28.2 b	22.3 b
			Tilt 0.29 l ha-1	26.4 b	20.3 b
Check	4.3 a	4.3 a	Check	58.6 a	58.6 a

Table 3. Effects of fungicides and fungicide application timing (Zadoks growth stage GS 31 versus GS 37 or GS 39) on foliar disease severity in winter wheat cv. Millennium in field experiments conducted in Nebraska, USA in 2006 and 2007. Means followed by the same letter within a column are not significantly different according to Fisher's least significant difference test at P = 0.05. Means with an asterisk within a row in a year are significantly different according to Fisher's least significant difference test at P = 0.05.

#### 4.4.3 Effects of fungicides and fungicide application timing on yield increase

In 2006, yield increase due to fungicide application was low and did not significantly differ among fungicides in both application timings. In 2007, yield increase due to fungicide application was much higher than in 2006, but also did not significantly differ among fungicides in both application timings (Table 4). Yield increase did not significantly differ between fungicide application timings in 2006. However, in 2007, fungicide application timing had a significant effect on yield increase for the fungicides Headline, Quilt, and Tilt (Table 4), and when averaged across fungicides (Fig. 3), with the GS 39 timing resulting in a higher yield increase than the GS 31 timing. The reason for the higher yield increase in the GS 39 timing compared to the GS 31 timing may be explained by the protection provided to the flag leaf which contributes significantly to yield (Ali et al., 2010; CiuHua et al., 2010; Rawson et al., 1983). In a GS 31 application, the residual fungicide activity would have waned by GS 39 and therefore would not provide the same level of protection to the flag leaf as a GS 39 application. Cook et al. (1999) showed that fungicide application to winter wheat at the GS 37 growth stage to control powdery mildew resulted in significantly higher yield than application at GS 33.



Fig. 2. Effect of fungicide application timing (Zadoks growth stage GS 31 versus GS 37 or GS 39), averaged across fungicides, on disease severity in winter wheat cv. Millennium in experiments conducted in Nebraska, USA in 2006 and 2007. Bars with an asterisk within a year are significantly different according to Fisher's least significant difference test at

2006			2007	2007		
Fungicide treatment	Yield increase (kg ha <sup>-1</sup> ) GS31 timing	Yield increase (kg ha <sup>-1</sup> ) GS37 timing	Fungicide treatment	Yield increase (kg ha <sup>-1</sup> ) GS31 timing	Yield increase (kg ha <sup>-1</sup> ) GS39 timing	
Headline 0.58 l ha-1	290 a	451 a	Headline 0.66 l ha-1	1370 a*	1585 a*	
Quadris 0.58 l ha-1	375 a	279 ab	Quadris 0.88 l ha <sup>-1</sup>	1332 a	1430 a	
Quilt 0.58 l ha <sup>-1</sup>	518 a	424 a	Quilt 1.02 l ha-1	1123 a*	1518 a*	
Stratego 0.73 l ha-1	474 a	339 a	Stratego 0.73 l ha-1	1156 a	1350 a	
			Tilt 0.29 l ha-1	1229 a*	1600 a*	
Check	0 a	0 b	Check	0 b	0 b	

Table 4. Effects of fungicides and fungicide application timing (Zadoks growth stage GS 31 versus GS 37 or GS 39) on yield increase in winter wheat cv. Millennium in field experiments conducted in Nebraska, USA in 2006 and 2007. Means followed by the same letter within a column are not significantly different according to Fisher's least significant difference test at P = 0.05. Means with an asterisk within a row in a year are significantly different according to Fisher's least at P = 0.05.

P = 0.05.



Fig. 3. Effect of fungicide application timing (Zadoks growth stage GS 31 versus GS 37 or GS 39), averaged across fungicides, on yield increase in winter wheat cv. Millennium in experiments conducted in Nebraska, USA in 2006 and 2007. Bars with an asterisk within a year are significantly different according to Fisher's least significant difference test at P = 0.05.

#### 4.4.4 Effects of fungicides and fungicide application timing on net return

In 2006, net return from fungicide application was very low. It ranged from \$-5 ha-1 to \$39 ha-1 in the GS 31 timing and from \$-14 ha-1 to \$25 ha-1 in the GS 39 timing and did not significantly differ from zero or among fungicides (Table 5). In 2007, net return from fungicide application was significantly higher than zero and ranged from \$148 ha-1 to \$191 ha-1 in the GS 31 timing and from \$177 ha-1 to \$239 ha-1 in the GS 39 timing (Table 5). Although net return did not significantly differ among fungicides, Headline resulted in the highest return in the GS 31 application timing whereas Tilt resulted in the highest return in the GS 39 application timing. The effect of application timing on net return was not significant for any fungicide in 2006. However, in 2007 it was significant for Headline, Quilt, and Tilt (Table 5), and when averaged across fungicides (Fig. 4), with the GS 39 application timing resulting in a higher net return than the GS 31 application timing. As explained above for yield increase, the higher net return in the GS 39 timing compared to the GS 31 timing is attributable to protection provided to the flag leaf by a GS 39 fungicide application. The probability of a positive net return was 0.63 and 1.00 in 2006 and 2007, respectively. It should be noted, however, that positive net returns in 2006 were very small compared to 2007.

2006			2007		
Fungicide treatment	Net	Net	Fungicide treatment	Net	Net
	return	return		return	return
	(\$ ha-1)	(\$ ha-1)		(\$ ha-1)	(\$ ha-1)
	GS31	GS37		GS31	GS39
	timing	timing		timing	timing
Headline 0.58 l ha <sup>-1</sup>	-5 a	19 a	Headline 0.66 l ha-1	191 a*	229 a*
Quadris 0.58 l ha-1	0 a	-14 a	Quadris 0.88 l ha-1	160 a 🦳	177 a
Quilt 0.58 l ha-1	39 a	25 a	Quilt 1.02 l ha-1	148 a*	218 a*
Stratego 0.73 l ha-1	24 a	5 a	Stratego 0.73 l ha-1	159 a	193 a
••••			Tilt 0.29 l ha-1	173 a*	239 a*
Check	0 a	0 a	Check	0 b	0 b

Table 5. Effects of fungicides and fungicide application timing (Zadoks growth stage GS 31 versus GS 37 or GS 39) on net return in winter wheat cv. Millennium in field experiments conducted in Nebraska, USA in 2006 and 2007. Means followed by the same letter within a column are not significantly different according to Fisher's least significant difference test at P = 0.05. Means with an asterisk within a row in a year are significantly different according to Fisher's least significantly different according to Fisher's least significant difference test at P = 0.05.



Zadoks growth stage

Fig. 4. Effect of fungicide application timing (Zadoks growth stage GS 31 versus GS 37 or GS 39), averaged across fungicides, on net return in winter wheat cv. Millennium in experiments conducted in Nebraska, USA in 2006 and 2007. Bars with an asterisk within a year are significantly different according to Fisher's least significant difference test at P = 0.05.

#### 4.4.5 Relationship between disease severity and yield increase

By applying different fungicides at two growth stages, different levels of disease were generated which resulted in corresponding yield increases. Thus, in 2007 when environmental conditions favored disease development, data were generated and used to model the relationship between disease severity and yield increase using linear regression analysis. This relationship can be used to estimate the yield increase to expect from a certain level of disease control from fungicide application. The results showed a significant, linear inverse relationship ( $r^2 = 0.56$ , p = 0.0122) between disease severity and yield increase (Fig. 5), implying that higher yield increases were realized in plots with lower disease severity and vice versa. Disease severity explained 56% of the variation in yield increase. Every unit of disease severity reduction resulted in a yield increase of 32.9 kg ha<sup>-1</sup>. This result demonstrates the potential for a yield benefit if a fungicide is applied to winter wheat when environmental conditions favor disease development.



Fig. 5. Relationship between disease severity and yield increase due to fungicide application in winter wheat cv. Millennium. Data were obtained from field experiments conducted in Nebraska, USA in 2007.

#### 4.4.6 Relationship between disease severity and net return

Net return from fungicide application was linearly and inversely related to disease severity (Fig. 6), which mirrored the relationship between yield increase and disease severity. However, the relationship between net return and disease severity was weaker ( $r^2 = 0.32$ , p = 0.0658) than the relationship between yield increase and disease severity. This was because factors that do not directly affect yield, such as fungicide and fungicide application costs, were used to calculate net return. Nevertheless, this result shows that when environmental conditions favor disease development, a higher level of disease control will result in a higher net return.



Fig. 6. Relationship between disease severity and net return due to fungicide application in winter wheat cv. Millennium. Data were obtained from field experiments conducted in 2007 and economic analyses conducted in 2009-2010 in Nebraska, USA.

#### 4.4.7 Relationship between yield increase and net return

In 2007, there was a strong, positive linear relationship between yield increase and net return (Fig. 7). Eighty five percent of the variation in net return was explained by yield increase. Every unit (kg ha<sup>-1</sup>) of yield increase resulted in a net return of \$0.17 ha<sup>-1</sup>.



Fig. 7. Relationship between yield increase and net return due to fungicide application in winter wheat cv. Millennium. Data were obtained from field experiments conducted in 2007 and economic analyses conducted in 2009-2010 in Nebraska, USA.

It should be noted, however, that profitability from fungicide application is dependent on many factors, including weather conditions favorable to disease development, the level of disease intensity during the growing season, the price of wheat, fungicide and fungicide application costs, fungicide application rates and timing, cultivar resistance, and cultural practices.

#### 5. Conclusions

We conclude that the fungicides Quilt, Headline, Tilt, Quadris, and Stratego effectively controlled foliar fungal diseases in winter wheat, resulting in yield increase and a profitable net return in 2007. Environment had a significant effect on yield increase and net return. In 2006 when dry conditions led to development of low levels of disease, yield increase and net return were very low. However, in 2007 when excessively wet weather favored development of high levels of disease, yield increase and net return were high. These results suggest that fungicide application to winter wheat can be profitable when environmental conditions favor development of damaging levels of disease. Timing of fungicide application at GS 39 generally resulted in a higher yield increase and a higher net return than a GS 31 timing. Therefore, under Nebraska conditions, when a farmer can afford only one spray in a growing season, spraying at GS 39 or later would likely be more beneficial than spraying earlier. Regression analysis of 2007 data showed an inverse linear relationship between yield increase and disease severity and between net return and disease severity, and a positive linear relationship between yield increase and net return, confirming the negative effect of disease on yield and suggesting a potential benefit from fungicide application to control foliar fungal diseases in winter wheat when environmental conditions favor the development of damaging levels of disease.

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