MANAGING WINTER WHEAT DISEASES IN WISCONSIN

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Introduction

Since 2010 in Wisconsin, several diseases of winter wheat have become extremely impactful. The first is stripe rust, caused by the fungal plant pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*). Stripe rust has been an increasing problem in the central Great Plains and areas of the upper Midwest due to milder winters (Chen, 2005). The second disease is Fusarium head blight (FHB), which is caused by several species in the *Fusarium graminearum* species complex. The last important epidemic of FHB in Wisconsin, occurred in 2015. This epidemic caused considerable issues in the feed supply due to grain being contaminated with mycotoxins. This resulted in financial losses for farmers around the state, due to dockage at the elevator.

The occurrence of both stripe rust and FHB in winter wheat in Wisconsin results in much confusion about proper disease management. Unfortunately, management practices can differ for both diseases on winter wheat. Furthermore, changes in the epidemiology of *Pst* and the species complex of *Fusarium* have added to this confusion. In the spring of 2017, the University of Wisconsin-Madison Field Crops Pathology laboratory documented several cases where *Pst* was able to overwinter on winter wheat in Wisconsin. Prior to this finding, it was assumed that *Pst* had to be transported via spores on air currents from southern states to initiate epidemics each season. Overwintering of *Pst,* may create a challenge for Wisconsin winter wheat farmers, as this could result in earlier and more severe epidemics.

The University of Wisconsin-Madison Field Crops Pathology laboratory has also conducted surveys of winter wheat fields in Wisconsin to document the *Fusarium* species complex affecting winter wheat. We identified more than one species of *Fusarium* affecting wheat (Mueller et al., 2018). This may lead to an increase challenge in management of FHB on wheat in Wisconsin. To better understand this challenge, knowledge of the *Fusarium* population is needed. Specifically, understanding the chemotype (mycotoxin signature) of *Fusarium* isolates collected from winter wheat fields in Wisconsin will help pathologists develop better management recommendations. Species within the *F. graminearum* complex produce deoxynivalenol (DON or vomitoxin) and forms derivatives of the compound based on its acetylation cites. The fungus is profiled into chemotypes based on these derivatives; 3 acetyldeoxynivalenol (3ADON) chemotype, 15-acetyldeoxynivalenol (15ADON) chemotype, and nivalenol (NIV) chemotype. The challenges related to these pathogens and the diseases they cause has led to research to address the following objectives.

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Objectives

1. Evaluate stripe rust-resistant cultivars and fungicide timings (integrated management) in the wheat-growing region of Wisconsin for control of stripe rust.
2. Identify the primary chemotype of the *Fusarium* species complex in Wisconsin and understand the impact chemotype has on isolate aggressiveness on winter wheat.

Methods

Stripe rust integrated management study. The experimental design was a 3 x 2 x 5 factorial arranged in a randomized complete block with four replicates per treatment. Three soft red winter wheat cultivars, which consisted of susceptible cultivar 'Pro Seed 420', moderately susceptible cultivar 'Kaskaskia', and resistant cultivar 'Pro Seed 380'. Cultivars were selected based on disease assessment from Wisconsin winter wheat variety trials in 2015 (data not shown). Two fungicide products were chosen to be applied to the wheat cultivars. These included: prothioconazole (0.21 kg/L active ingredient; a.i.) + tebuconazole (0.21 kg/L a.i.) as the formulated product Prosaro 421 SC® (Bayer Crop Science Inc., Leverkusen, Germany) and Pyraclostrobin (0.25 kg/L a.i.) as the formulated product Headline SC (BASF Chemical Company, Research Triangle Park, NC). Pyraclostrobin was applied at a rate of 0.66 L/ha and prothioconazole + tebuconazole was applied at 0.48 L/ha. Five fungicide application programs were chosen. Fungicides were applied at Feekes 6, Feekes 8, and Feekes 10. To compare these single application programs to the best possible fungicide protection scenario, an additional full-season fungicide program was also implemented where fungicide was applied at Feekes 6, Feekes 8, and Feekes 10 and Feekes 10.5.1. Pyraclostrobin was used for Feekes 6-10 applications, while prothioconazole + Tebuconazole was used for Feekes 10.5.1 applications. Finally, a non-treated control was included to round out the five fungicide application programs. Feekes 6 fungicide applications were applied on April 18 in 2016 and April 24 in 2017. Feekes 8 fungicide applications were applied on May 13, 2016 and May 19, 2017. Feekes 10 applications applied on May 20, 2016 and May 25, 2017. Feekes 10.5.1 fungicide applications were made on June 1, 2016 and June 4, 2017.

Winter wheat plots were planted on September 24, 2015 for the 2016 growing season and on September 27, 2016 for the 2017 wheat crop. Plots were 6.4 m long and 2.3 m wide with 1.2-m alleys between plots in 2016. In 2017, plots were and 6.1 m long and 2.3 m wide with 1.2-m alleys between plots. In both years the seeding rate was 3.75 million seeds/ha. Urea was applied at 140 kg/ha in early spring according to local recommendations. Natural sources of pathogen inoculum were relied upon for disease. Fungicides were applied using a CO_2 pressurized backpack sprayer equipped with TTJ60-11002 Turbo TwinJet flat fan nozzles calibrated to deliver 187 l/ha at 30psi. Plots were harvested on July 19 in 2016 and August 1 in 2017.

Stripe rust was evaluated by visually estimating average disease incidence (% plants with symptoms) and average disease severity (% flag leaf covered by stripe rust symptoms) per plot with the aid of standardized area diagrams. Plots were rated for disease on Jun 6 and again on Jun 17 in 2016. Ratings for 2017 were on Jun 9 and again on Jun 21. Disease index (DX) was calculated by converting disease incidence (DI) and disease severity (DS) to proportions and then multiplying them together (DX = DI x DS; Zeng and Luo, 2006). Yield was determined by harvesting the center 1.5 m width of the entire length of each plot using an Almaco (Nevada, IA) SPC40 small-plot combine equipped with a HarvestMaster (Logan, UT) HM800 Classic Grain gauge.

***Fusarium* isolate collection and chemotype identification.** Wheat heads were collected in 12 counties throughout Wisconsin during the 2016-2017 growing seasons. Locations were chosen with an emphasis of targeting major winter wheat growing areas. Specifically, 1-2 wheat fields per county were sampled each year, arbitrarily collecting 10 FHB symptomatic heads per

field while walking in a Z-pattern. In total, 10-20 heads were collected per county per year with GPS coordinates recorded at the field level.

Five kernels per head were surface sterilized in one-minute intervals with 95% ethanol, sterile distilled water, and 1% sodium hypochlorite, then rinsed in sterile distilled water and dried on filter paper. Kernels were then placed on potato dextrose agar (PDA) with ampicilin (250mg/ml), rifampicin (10mg/ml), and streptomycin (20mg/ml) in order to trigger mycelial growth. Antibiotics were incorporated into the sterilized PDA media before pouring into Petri plates. After 4-5 days of growth, one plug (5mm) per Petri plate was transferred onto smaller Petri plates containing PDA. This transfer resulted in what was considered a single-spored isolate. Once isolates grew to a diameter of approximately 50 mm, plugs were transferred into petri dishes filled with potato dextrose broth (PDB) and placed on the lab bench to grow. Mycelial mats were grown for 7-10 days at room temperature and vacuum filtrated to collect mats as material for DNA extractions. Extractions were performed using FastDNA kit (MP Biomedicals, Irvine, CA) according to the manufacturer's instructions. *Fusarium graminearum*-specific PCR multiplex primer set 3CON/3NA/3D15A/3D3A was used to confirm species and chemotype of *Fusarium* for each isolate. Amplified fragments were anticipated to be 840, 610, or 243 bp corresponding to the nivalenol (NIV), 15ADON, and 3ADON chemotypes, respectively (Starkey et al. 2007).

Greenhouse aggressiveness evaluation. From the 146 isolates positively chemotyped in 2016, isolates were grouped by county and three isolates were arbitrarily selected for each location in Wisconsin. However, 2 isolates from Monroe County were misidentified as *F. graminearum* and were removed from the study. This led to a total of 31 single-spore isolates from 11 counties used in this study.

A greenhouse experiment was conducted to test the aggressiveness of 29 *Fusarium spp.* with 15ADON chemotype and 2 *Fusarium* spp. with the 3ADON chemotype on the susceptible winter wheat cultivar 'Hopewell'. Seed was vernalized and planted (1" in depth) in Ray Leach "Cone-tainer"™ (Stuewe & Sons, Inc., Tangent, Oregon, USA). To vernalize winter wheat, seeds were surface-disinfested in 95% ethanol and 0.06 % sodium hypochlorite for one minute each. Seeds were then washed 3 times in deionized H_2O. Seeds were placed evenly on moistened filter paper in a petri plate and wrapped in parafilm. Plates were stored in refrigerator at 4 °C with no light for >6 weeks. Procedures were based on work done by Ördög and Molnár (2011) and Sasani et al. (2009).

Soil medium consisted of 10 shovelfuls of sterilized field soil to 1 bag of Pro Mix HP potting soil (ProMix Technologies LLC, Rockwall, Texas) and Nutricote Total type 100 blend slow release fertilizer (Arysta Lifescience America, Broadway, New York) containing a 13:13:13 ration of nitrogen (N), phosphorus (P), and potassium (K). A piece of paper towel was placed in the bottom of each Cone-tainer to reduce soil loss and pots were filled with 1-2 inches remaining empty at the top for watering. Plants were grown at day temperature of 22-25 °C and night temperature of 20-21 °C with a 12-hour photoperiod. Plants were irrigated daily until anthesis and fertilized weekly with Peter's (20:10:20, N:P:K) (Everris NA Inc., Dublin, Ohio) after leaf emergence.

While wheat was being grown to head emergence, single-spore *Fusarium* isolates were grown in 25ml of CMC media in a 125ml Erlenmeyer flask and placed on a shaker at room temperature for 6 days to prepare spores for inoculation. Spores were held at 4 °C until isolates were ready for inoculation. Spore concentrations were quantified with a hemacytometer and final inoculum was diluted to 1 x 10^5 macroconidia/ml with deionized water. Diluted inoculum was used to inoculate wheat spikes using the single-floret injection method. The central floret of a spikelet at anthesis was inoculated with 10 µl of inoculum using a pipette and denoted with non-toxic marker. Wheat heads of separate plants of the same cultivar were inoculated with 10 ul of deionized water and served as a non-treated control. Inoculated heads were covered with a plastic bag to promote infection, which was removed 3 days after inoculation. Disease measurements

were taken 7, 10 and 14 days after inoculation (dai) by measuring symptomatic area on blighted spikelets using a digital caliper. Two repetitions of a single factor (isolate) experiment were conducted in a greenhouse. A randomized complete block design (RCBD) was used with four replications per isolate.

Results and Discussion

Stripe rust integrated management study. Yield and disease levels varied considerably in the 2016 and 2017 seasons. Cultivar and fungicide treatment main effects on yield and DX were significant ($P < 0.001$) (Table 1). There was no interaction of cultivar x fungicide treatment for yield ($P > 0.05$), however DX did result in a significant cultivar x fungicide treatment interaction in both 2016 and 2017 *(P*<0.001). In 2016, Pro Seed 380 and Pro Seed 420 had significantly higher yields than Kaskaskia (Table 1). However, Pro Seed 380 had significantly ($P < 0.001$) lower DX compared to Pro Seed 420 and Kaskaskia (Table 1). In 2017, Pro Seed 380 had the lowest DX and significantly higher yield than Pro Seed 420 and Kaskaskia (Table 1).

In 2016, full-season fungicide application led to significantly lower DX than the non-treated control for Kaskaskia and Pro Seed 420 (Fig. 1). There were no significant differences between treatments for Pro Seed 380. Pyraclostrobin and prothioconazole + tebuconazole applied at Feekes 8 and 10 resulted in no significant differences for DX ($P > 0.05$) when compared to the full-season fungicide application for Kaskaskia and Pro Seed 420 (Fig. 1). Feekes 6 applications of both pyraclostrobin and prothioconazole + tebuconazole resulted in no significant differences in DX compared to the non-treated control. However, Feekes 6 applications of prothioconazole + tebuconazole resulted in no difference in DX compared to full-season fungicide application on Kaskaskia.

In 2017, full season application of fungicide on Kaskaskia resulted in a 182% decrease in DX compared to the non-treated control; while DX was reduced by 184% for the same treatment compared to not treating Pro Seed 420 (Fig. 2). For Pro Seed 420, pyraclostrobin applied at Feekes 8 and 10 and prothioconazole + tebuconazole at Feekes 8 resulted in DX comparable to full-season application of fungicide. Pyraclostrobin and prothioconazole + tebuconazole applications at Feekes 6 and prothioconazole + tebuconazole at Feekes 10 differed from full-season application of fungicide, resulting in significantly lower DX than the non-treated control (Fig. 2). Applications of pyraclostrobin and prothioconazole + tebuconazole at Feekes 8 and Feekes 10 on Kaskaskia were comparable to the full-season application of fungicide. Feekes 6 application using pyraclostrobin was not different from not treating. No fungicide treatment differences were observed on the resistant cultivar Pro Seed 380 (Fig. 2).

Full season fungicide application led to the highest yields across all cultivars for the 2016 and 2017 field seasons (Fig. 3 A and B). In 2016 all other treatments were not different from the non-treated control (Fig. 3 A). However, pyraclostrobin applied at Feekes 10 and Feekes 8 resulted in comparable yields to full-season fungicide application (Fig. 3 A). In 2016, single applications of pyraclostrobin at Feekes 8 and Feekes 10 was comparable to the full-season fungicide program, which led to a 6% increase in yield.

In 2017, all fungicide treatments resulted in significantly higher yields compared to not treating, except for prothioconazole + tebuconazole applied at Feekes 8 (Fig. 3 B). Furthermore, only pyraclostrobin applied at Feekes 8 resulted in comparable yield (5896.8 kg/ha) to the full-season fungicide application. Pyraclostrobin applied at Feekes 8 produced a difference of 628.8 kg/ha when compared to not-treating which was a 11.2% yield increase (Fig. 3 B). Full-season fungicide coverage in both seasons led to a yield increase of 12-18% compared to the non-treated control (Fig. 3 A and B).

Table 1. Disease index and yield for three soft red winter wheat varieties grown in Wisconsin in 2016 and 2017.

Variety	2016		2017	
	Disease Index (DX)[y,x]	Yield (kg/ha)[x]	Disease Index (DX)[y,x]	Yield (kg/ha)[x]
Kaskaskia	0.01572 b	6598.98 b	0.03 a	5846.19 b
Pro Seed 380	0.01018 c	7022.20 a	0.01 b	6173.62 a
Pro Seed 420	0.02121 a	7044.51 a	0.03 a	5361.35 c

[y]Disease index (DX) was calculated taking proportional values of disease incidence (DI) multiplied by disease severity (DS), (DX=DI x DS).
[x]Means followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; α=0.05).

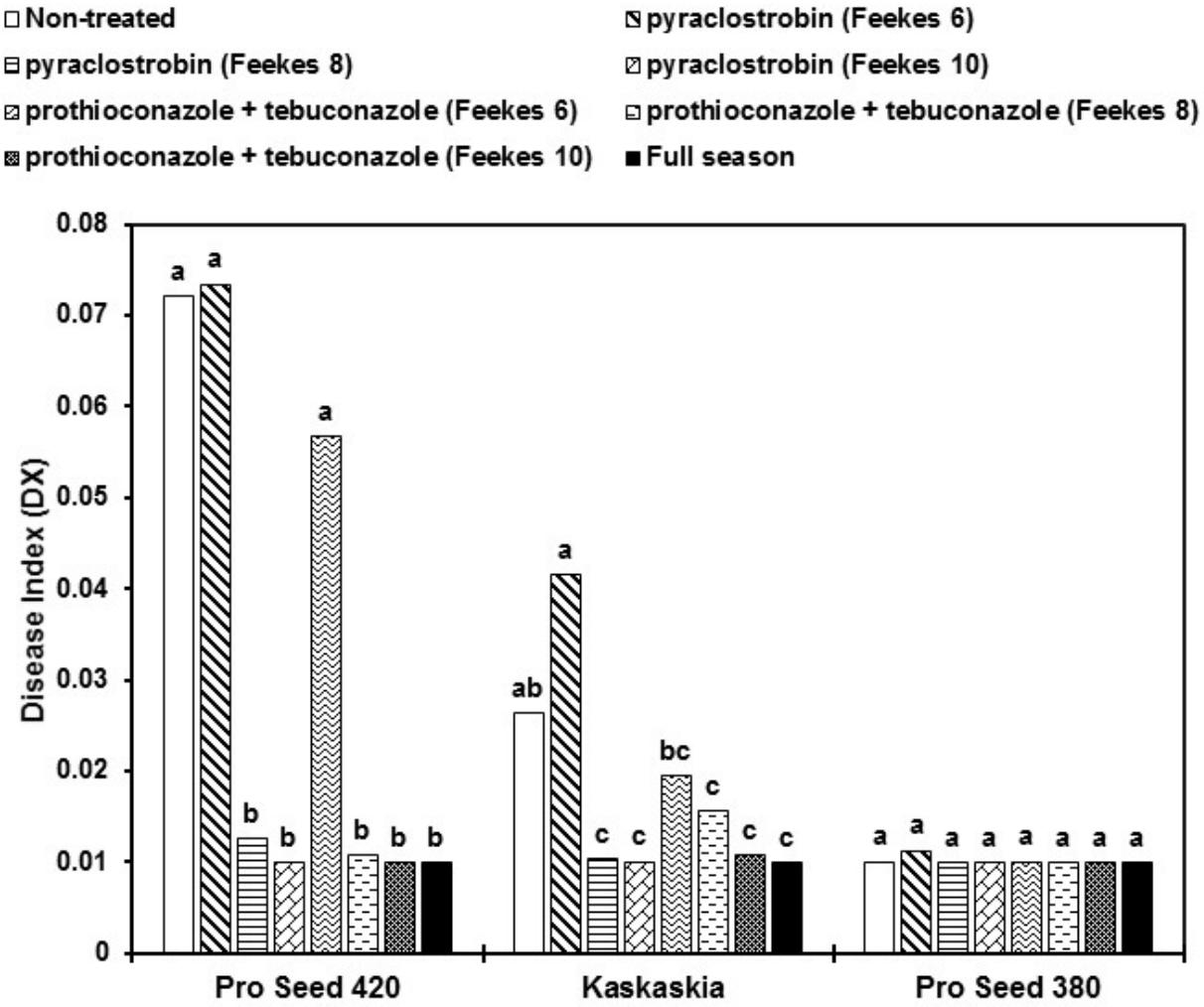

Figure 1. Mean disease index (DX) for each fungicide regime applied to three different soft red winter wheat cultivars in Arlington Wisconsin in 2016. Disease index (DX) was calculated taking proportional values of disease incidence (DI) multiplied by disease severity (DS), (DX=DI x DS).

Means followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; α=0.05).

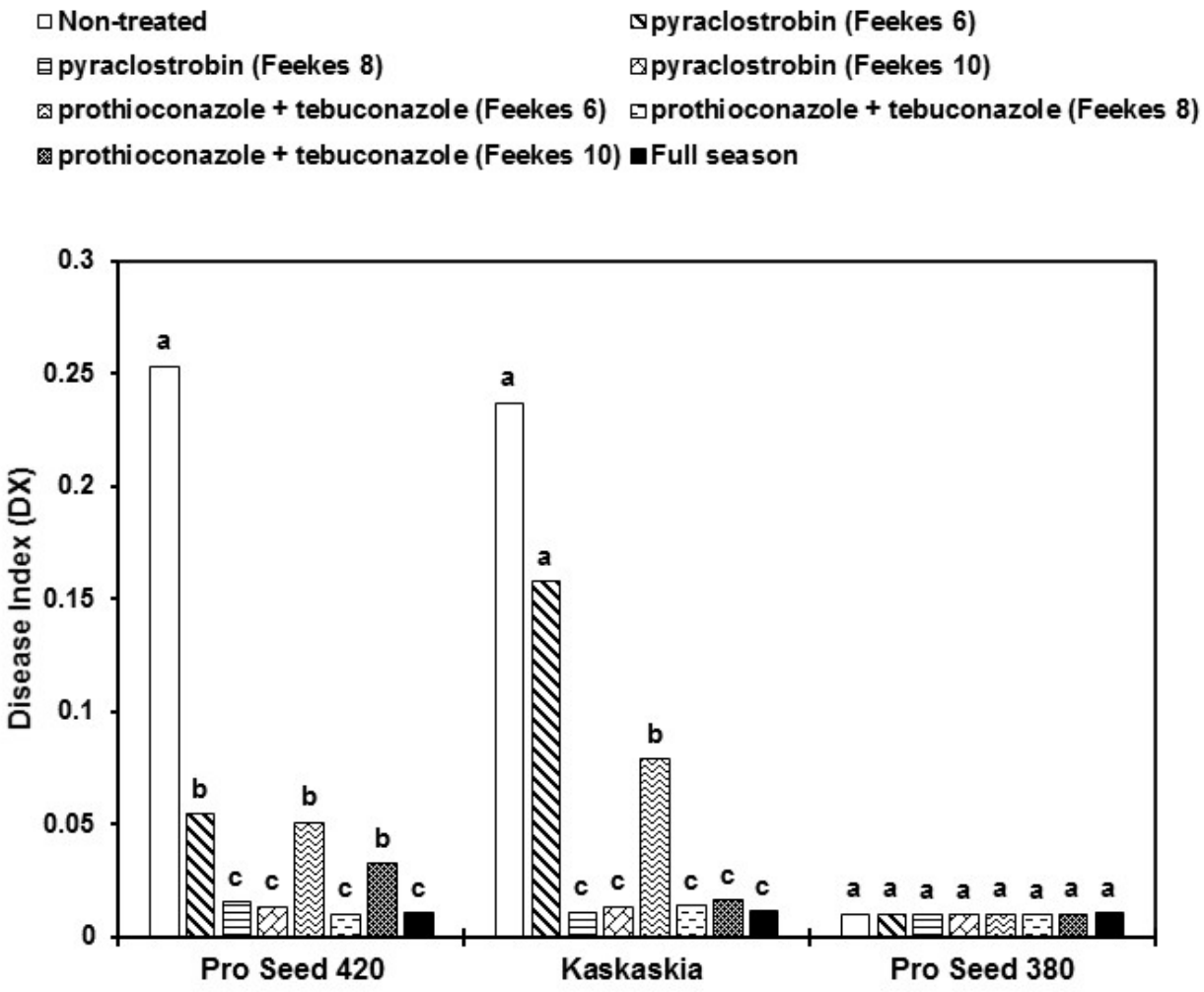

Figure 2. Mean disease index (DX) for each fungicide regime applied to three different soft red winter wheat cultivars in Arlington Wisconsin in 2017. Disease index (DX) was calculated taking proportional values of disease incidence (DI) multiplied by disease severity (DS), (DX=DI x DS). Means followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; α=0.05).

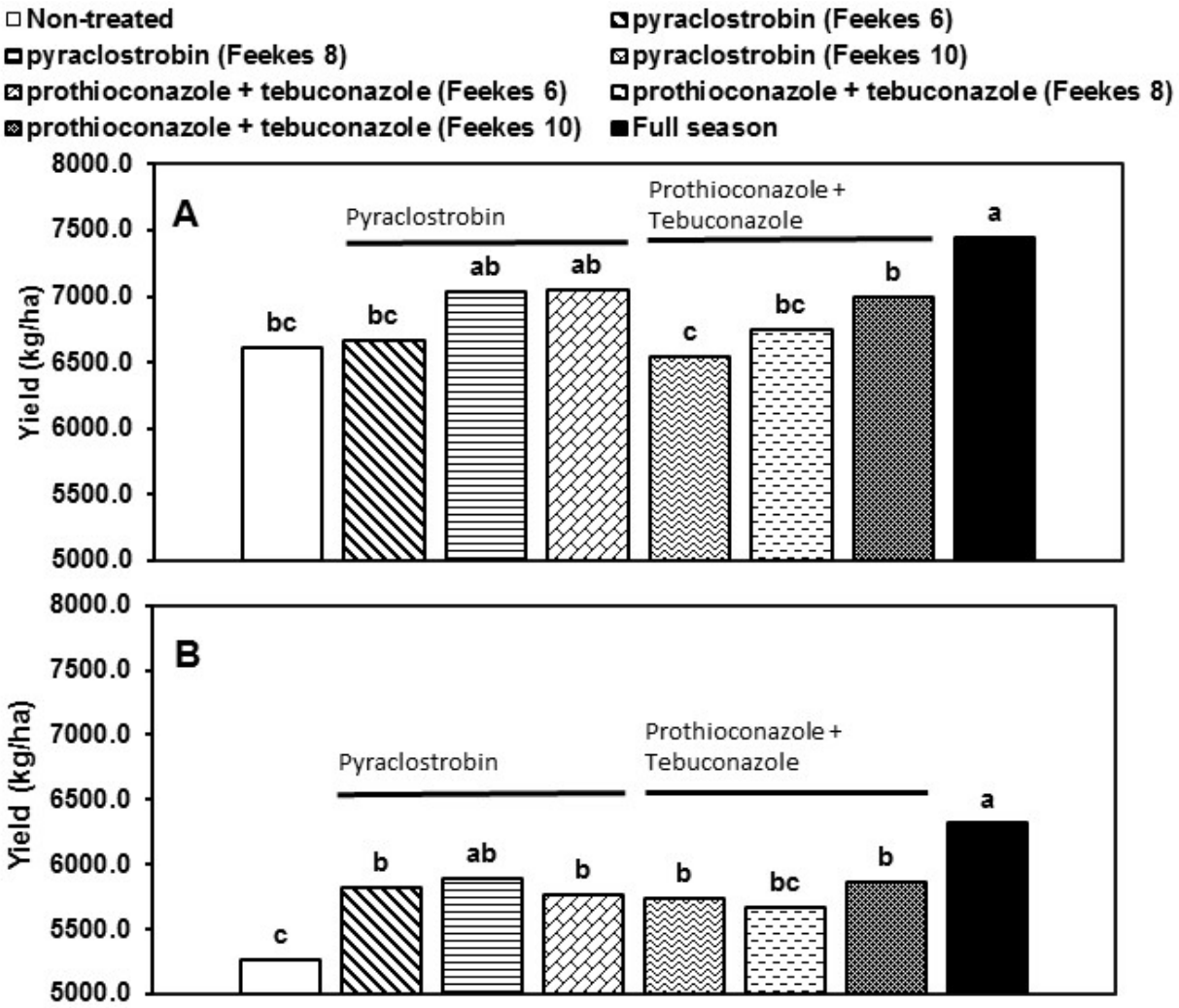

Figure 3. Yield of soft red winter wheat grown in Arlington Wisconsin and subjected to various fungicide application regimes in **A,** 2016 and **B,** 2017. Means followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; α=0.05).

***Fusarium* spp. identification and chemotyping.** Among 195 wheat heads collected in 2016 in Wisconsin, 145 *Fusarium* spp. were positively chemotyped as 3ADON and 15ADON. The 15ADON chemotype was predominant and amounted to 90% of the isolates, while 10% of isolates were identified as the 3ADON chemotype. In 2017, 185 samples were collected and 120 of them were positively chemotyped. Similar to 2016, 92% of the isolates were identified as the 15ADON chemotype while 8% were the 3ADON chemotype. The 3ADON chemotypes were found in six counties in 2016 and five counties in 2017. Interestingly, all 3ADON isolates belonged to counties found in the northern half of Wisconsin. Additionally, sequencing of 3ADON isolates DO12 and DO14 from the aggressiveness study identified isolates as *F. culmorum.* A total of five *F. culmorum* isolates with the 3ADON chemotype were identified in 2016. All *F. culmorum* isolates were found in a single field located in Door Co.

Greenhouse aggressiveness evaluation. The 31 isolates from 2016 tested in the greenhouse were found to have significant differences in AUDPC values (P<0.0001) (Fig. 4). AUDPC values ranged from 90.5 to 304.4. Isolate CO6 produced the greatest level of disease but

was not significantly different from 11 other isolates. The 3ADON isolates, DO12 and DO14, were highly aggressive and were in the top 6 for AUDPC values (Fig. 4).

At the county level, there were significant differences between isolates from counties in Wisconsin ($P<0.0001$). Isolates from Door County had the highest disease level with an AUDPC value of 259.2 while isolates from Eau Claire County resulted in the lowest disease with a value of 167.8 (Fig. 5). Isolates from Door Co. resulted in disease levels that were not significantly different from isolates in 6 other counties. Isolates from Baron Co. and Walworth Co. resulted in low disease levels and were not statistically different from isolates from Eau Claire Co.

Separating counties into their eight statewide districts, isolates collected from east and south-central districts were the most aggressive with AUDPC values of 247.4 and 246.7, respectively (Fig. 6). The isolates collected from the northwest and west-central districts had the lowest disease levels. Isolates collected from the north-central, south-west and central districts resulted in disease levels that were not statistically different from those that resulted from isolates collected from east and south-central districts. Districts and counties with the most aggressive isolates fall into areas where majority of winter wheat is grown in Wisconsin.

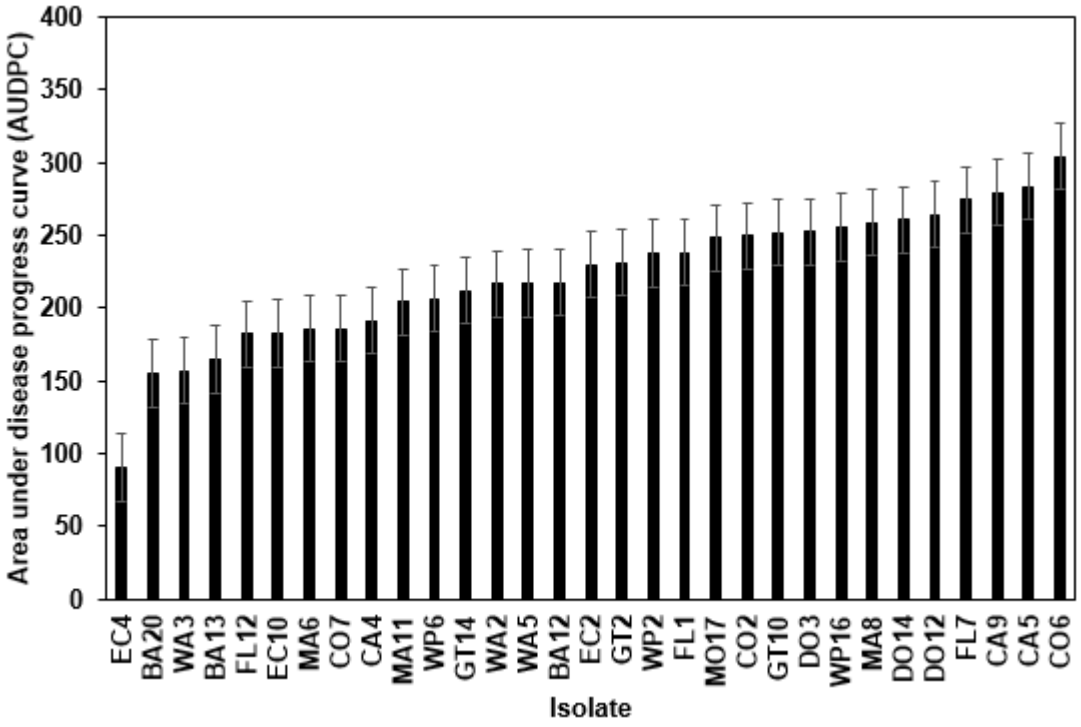

Figure 4. Area under disease progress curve (AUDPC), by isolate, for 29 *Fusarium* spp. with 15 ADON chemotype and 2 *Fusarium* spp. with the 3 ADON chemotype on the susceptible winter wheat cultivar 'Hopewell' in a greenhouse experiment.

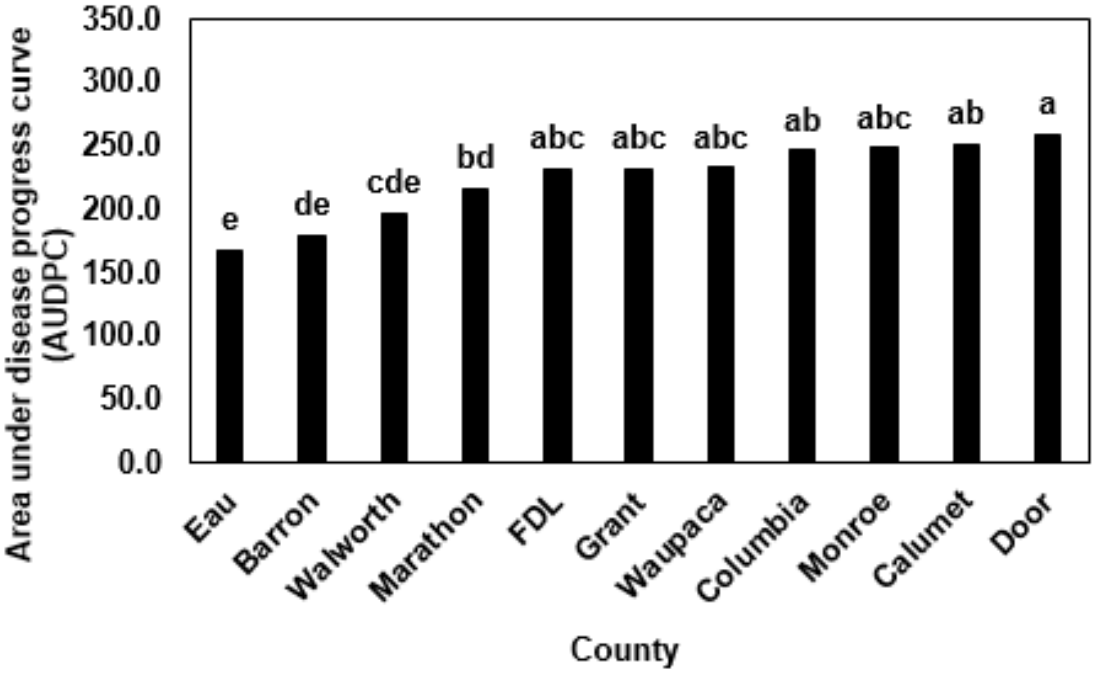

Figure 5. Area under disease progress curve (AUDPC), by county, for 29 *Fusarium* spp. with 15 ADON chemotype and 2 *Fusarium* spp. with the 3 ADON chemotype on the susceptible winter wheat cultivar 'Hopewell' in a greenhouse experiment. Bars with the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; α=0.05).

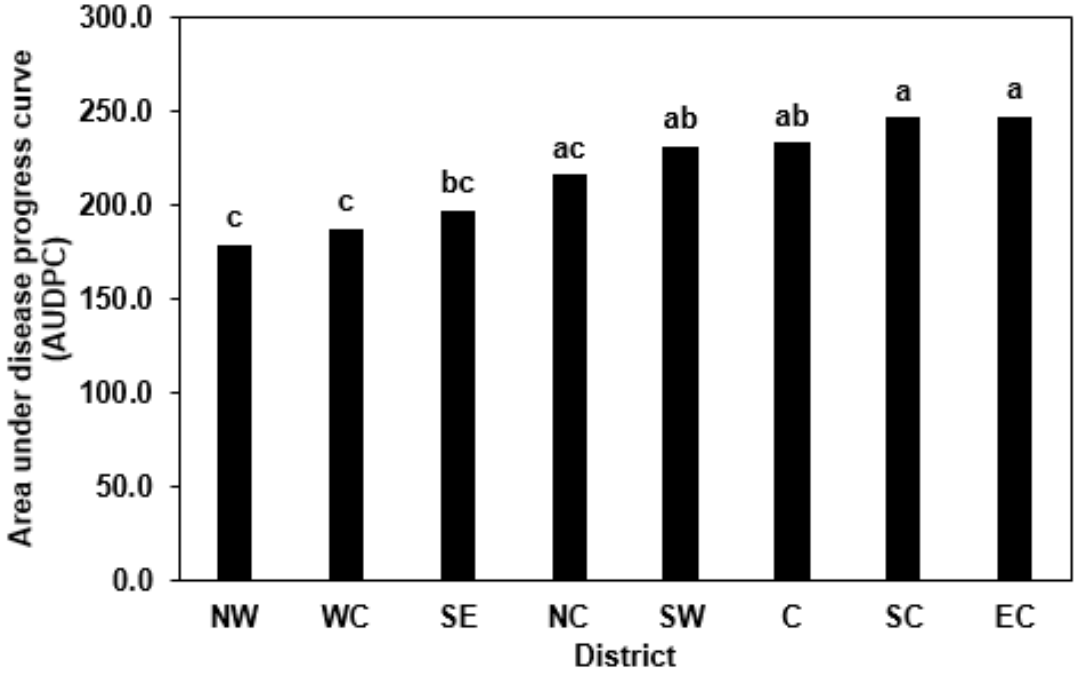

Figure 6. Area under disease progress curve (AUDPC), by district, for 29 *Fusarium* spp. with 15 ADON chemotype and 2 *Fusarium* spp. with the 3 ADON chemotype on the susceptible winter wheat cultivar 'Hopewell' in a greenhouse experiment. Bars with the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; α=0.05).

Summary

Planting resistant cultivars and applying foliar fungicides are common management practices to control for this pathogen. In this 2-year study, two fungicides applied at three growth stages were tested on three soft red winter wheat cultivars varying in levels of resistance to stripe rust. Both fungicides (prothioconazole + tebuconazole and pyraclostrobin) applied at Feekes 8 and 10 reduced disease index (DX) and increased yield compared with the non-treated control in susceptible (Pro Seed 420) and moderately resistant cultivars (Kaskaskia). The highly resistant cultivar (Pro Seed 380), had the highest yields and fungicide treatments had no effect on disease levels. This study confirmed that cultivar resistance to stripe rust can be highly effective in managing the disease, while a properly timed fungicide application can be critical on susceptible cultivars.

This study also assessed chemotype population from 2016 and 2017 in Wisconsin. Over both growing seasons, 91% of isolates were identified as the 15ADON chemotype while 9% of isolates were positively identified as the 3ADON chemotype. Aggressiveness was quantified by area under disease progress curve (AUDPC) over 14 days post inoculation, with AUDPC values ranging from 304.35 to 90.5. The most aggressive isolates were found to be located in the highest wheat production areas in the state. 3ADON isolates were among the most aggressive. These results show the baseline frequency and distribution of 3ADON and 15ADON chemotypes observed in Wisconsin,

which should be monitored in the future. This research also demonstrates that there is a wide range in *Fusarium* population in Wisconsin. This range in population can create challenges in managing FHB, especially considering commercial cultivars only have partial resistance to FHB. **Thus, farmers should focus on choosing winter wheat cultivars that are rated with the highest resistance to stripe rust they can find. With only marginal FHB resistance, in-season fungicide management should then focus on controlling FHB.** This integrated approach can help Wisconsin farmers be profitable while efficiently managing winter wheat diseases.

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