1 **Effects of Pre- and Post-Anthesis Applications of Demethylation Inhibitor Fungicides on**

2 **Fusarium Head Blight and Deoxynivalenol in Spring and Winter Wheat**

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21 **Abstract**

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1   Anthesis is generally recommended as the optimum growth stage for applying a foliar fungicide to

2   manage Fusarium head blight (FHB) and the *Fusarium*-associated toxin, deoxynivalenol (DON), in

3   wheat. However, since it is not always possible to treat fields at anthesis, studies were conducted to

4   evaluate pre- and post-anthesis treatment options for managing FHB and DON in spring and winter

5   wheat. Network meta-analytical models were fitted to data from 19 years of fungicide trials, and log

6   response ratio ($\bar{L}$) and approximate percent control ($\bar{C}$) relative to a nontreated check were estimated as

7   measures of the effects of six treatments on FHB index (IND: mean percentage of diseased spikelets per

8   spike) and DON. The evaluated treatments consisted of either Caramba (metconazole) applied early (at

9   heading; CE), at anthesis (CA), or late (5 to 7 days after anthesis; CL), or Prosaro (prothioconazole +

10   tebuconazole) applied at the same three times and referred to as PE, PA, and PL, respectively. All

11   treatments reduced mean IND and DON relative to the nontreated check, but the magnitude of the effect

12   varied with timing and wheat type. CA and PA resulted in the highest $\bar{C}$ valued for IND, 52.2% and

13   51.5%, respectively, compared to 45.9% for CL, 41.3% for PL and less than 33% for CE and PE.

14   Anthesis and post-anthesis treatments reduced mean IND by 14.9 to 29.7% relative to pre-anthesis

15   treatments. The estimated effects size was also statistically significant for comparisons between CA and

16   CL and PA and PL; CA reduced IND by 11.7% relative to CL, whereas PA reduced the disease by

17   17.4% relative to PL. Differences in efficacy against IND between pairs of prothioconazole +

18   tebuconazole and metconazole treatments applied at the same timing (CE vs. PE, CA vs. PA, and CL vs.

19   PL) were not statistically significant. However, CA and CL outperformed PA and PL by 7 and 12.8%,

20   respectively, in terms of efficacy against DON. All application programs had comparable efficacy

21   against IND between spring and winter wheat types, but efficacy against DON was 10 to 16% greater

22   for spring than winter wheat for applications made at or after anthesis. All programs led to an increased

23   in mean grain yield and test weight relative to the nontreated check.

24   Fusarium head blight [(*Fusarium graminearum* Schwabe) (FHB)], an economically important

25   disease of small grain crops, and its associated mycotoxin, deoxynivalenol (DON), are best managed by

1 integrating strategies such as genetic resistance, a timely fungicide application, crop rotation, and tillage

2 (Blandino et al. 2012; Lori et al. 2009; McMullen et al. 2008, 2012; Willyerd et al. 2012). A quantitative

3 synthesis of data from more than 100 trials showed that when applied at anthesis, the demethylation

4 inhibitor (DMI) fungicides metconazole (Caramba; BASF Corp., Research Triangle Park, NC),

5 prothioconazole (Proline 480 SC; Bayer CropScience, Research Triangle Park, NC), and

6 prothioconazole + tebuconazole (Prosaro; Bayer CropScience, Research Triangle Park, NC) provide

7 between 45 and 52% control of FHB index and DON (Paul et al. 2008). Subsequent analyses showed

8 that the combination of an anthesis application of prothioconazole + tebuconazole and genetic resistance

9 resulted in more than 70% reduction of both FHB index and DON, relative to the nontreated, susceptible

10 check (Willyerd et al. 2012). Furthermore, the responses in this combination were additive in terms of

11 percent control.

12   Anthesis (Large 1954; Zadoks et al. 1974), characterized in wheat by the extrusion of anthers

13 from the florets of the spike, has been previously accepted as the optimum growth stage for fungicide

14 application for Fusarium head blight (FHB) control (Tateishi et al. 2014). Extrusion (Graham and

15 Browne 2009; Kubo et al. 2013; Lu et al. 2013), retention (Buerstmayr et al. 2013), and fertility (Wang

16 et al. 2015) of anthers, as well as flowering cleistogamy (Kubo et al. 2010) have all been shown to be

17 associated with cultivar susceptibility to FHB and disease intensity. The process of anthesis is divided

18 into three stages, early (Feekes 10.5.1; anthers extruded in the middle third of the spike [Zadoks GS60-

19 61]), mid (Feekes 10.5.2; anthers extruded in the upper third of the spike [Zadoks GS64-65]), and late

20 (Feekes 10.5.3; anthers extruded in the bottom third of the spike [Zadoks GS68-69]) anthesis. Temporal

21 variation is frequently observed in the development of tillers in the same field, with secondary tillers

22 taking from a few to several days after primary tillers to reach anthesis. Consequently, anthesis at the

23 plot or field scale is not a fixed point in time, but rather a window that varies in length from a few to

24 several days, depending on growing conditions, cultivar growing habit, crop production practices (row

25 spacing, planting density, and fertility program), and complex interactions among these factors.

1   However, since one has to define a fixed time during the anthesis window for treatment application,

2   anthesis is often considered to be reached when 50% of the primary tillers are at Feekes 10.5.1 (Zadoks

3   GS60-61). This is also a commonly used criterion for recommending fungicide sprays for FHB

4   management in commercial fields.

5   However, for practical reasons, producers have not always been able to treat fields at the

6   recommended early anthesis growth stage. For instance, since FHB is often most severe when it rains

7   during anthesis, field conditions are frequently not conducive to ground applications when fungicides

8   are most warranted. Although aerial application is a commonly-used alternative, the fact that this is

9   usually done by custom applicators means that treatments can be applied only when an aircraft becomes

10 available, which may be before or after anthesis. These limitations have led to questions regarding the

11 efficacy of pre- and post-anthesis fungicide applications. Answers to these questions will determine if

12 producers may have some flexibility in treating their fields either a few days before or after anthesis,

13 without compromising FHB and DON management.

14   A few individual studies have investigated fungicide application timings for FHB and DON

15 management in wheat, with results suggesting that the magnitude of the effect may depend on which

16 fungicide is applied and the conditions under which any fungicide applications are made. However,

17 most frequently, the highest levels of efficacy (percent control relative to a nontreated check) were

18 observed for anthesis applications. For instance, evaluating pre-anthesis treatments of prothioconazole

19 for FHB and DON management in winter wheat in the United Kingdom, Edwards and Godley (2010)

20 showed that although applications at stem elongation (Zadoks GS31 [Feekes 6]) or full flag leaf

21 emergence (Zadoks GS39 [Feekes 9]) reduced FHB incidence by 50 and 58% and DON by 27 and 49%,

22 respectively, the efficacy of a mid-anthesis (Zadoks GS65 [Feekes 10.5.2]) application was considerably

23 higher, being 83% for incidence and 57% for DON. Based on a similar study on hard red spring wheat

24 in Minnesota, Wiersma and Motteberg (2005) also reported that across several cultivars, tebuconazole

25 (Folicur 3.6 F; Bayer CropScience, Research Triangle Park, NC) was more effective at reducing FHB

1   when applied at early anthesis (Zadoks GS60 [Feekes 10.5.1]) than at GS39. Comparing anthesis and

2   post-anthesis applications of thiophanate-methyl (Topsin M; Nippon Soda Co. Ltd., Chiyodaku, Tokyo)

3   in Japan, Yoshida et al. (2012) observed that a single anthesis application reduced FHB severity by 82%

4   in 2005 and 36% in 2006, compared to untreated checks. Contrastingly, treatments applied at 10 days

5   after anthesis only reduced severity by 25% in the first year and 17% in the second, but the effect of this

6   treatment was not statistically significant.

7   In one of the first published studies to compare the effects of anthesis and post-anthesis

8   applications of the fungicides prothioconazole + tebuconazole and metconazole on FHB and DON in

9   soft red winter wheat in the US Midwest, D'Angelo et al. (2014) reported that post-anthesis treatments

10   were just as or more efficacious (based on percent control) than anthesis treatments, in both naturally

11   infected and inoculated trials. Based on a meta-analysis of data from field trials conducted in Ohio and

12   Illinois from 2011 to 2013, they observed that relative to a nontreated check, applications made two

13   days after anthesis (DAA) resulted in the highest percent control of both FHB index and DON, 69 and

14   54%, respectively, followed by applications at four (62 and 52%) and six (62 and 48%) DAA, and at

15   anthesis (56 and 50%). Post-anthesis applications reduced FHB index by up to 30%, relative to the

16   anthesis application, but for DON, the corresponding differences were not statistically significant. The

17   authors further observed that the greatest difference in efficacy between anthesis and post-anthesis

18   treatments occurred when conditions were cool in the days leading up to anthesis or when it rained

19   during the application of the anthesis treatments. This led them to hypothesize that weather conditions

20   between heading and early grain-fill affected efficacy by changing the length of the anthesis window

21   and flowering synchrony and that rainfall affected efficacy by reducing fungicide deposition and

22   coverage.

23   The results of the study by D'Angelo et al. (2014) led us to question whether the efficacy of

24   post-anthesis applications would vary between winter and spring wheats, given that in most northern

25   winter wheat growing regions the crop reaches anthesis in the spring when conditions are relatively

1   cool, whereas in spring wheat production regions anthesis is reached in the summer, and generally under

2   warmer conditions than those of winter wheat. Although the infection process and FHB development are

3   generally considered to be similar across growing regions and market classes, some aspects of

4   epidemiology and management of this disease-toxin complex have previously been shown to vary

5   between winter and spring wheats. For instance, results from studies conducted in the US showed that

6   wheat type (spring vs. winter) had a significant effect on the efficacy of several DMI fungicides

7   (including prothioconazole + tebuconazole and metconazole) against FHB index and DON (Paul et al.

8   2007, 2008), and that relationships between visual estimates of FHB and DON varied between spring

9   and winter wheat classes (Paul et al. 2005, 2006), as did functional relationships between FHB index

10   and grain yield (Madden and Paul 2009). Based on these findings and for reasons related to differences

11   in growing conditions between spring and winter wheats, we hypothesized that the effects of pre- and

12   post-anthesis applications on FHB and DON would vary between the wheat types and growing regions.

13   In this study we analyzed a data set consisting of 292 trials conducted over 19 years to revisit the

14   question of pre- and post-anthesis fungicide options for FHB and DON management in wheat. Focusing

15   on the two most effective DMI fungicides, metconazole and prothioconazole + tebuconazole (Paul et al.

16   2008), we have expanded on the work done by D'Angelo et al. (2014) by conducting a more

17   comprehensive analysis of data from a wider range of environments and wheat market classes. This has

18   allowed us to evaluate the efficacy and variability of FHB and DON responses to these fungicides, as

19   well as the effects of location- or region-specific factors on efficacy. The specific objectives were to: 1 -

20   quantify and compare the efficacy of pre- and post-anthesis applications of prothioconazole +

21   tebuconazole and metconazole against FHB and DON in terms of mean percent control relative to a

22   nontreated check, 2 - determine whether there was an overall gain or loss in efficacy with pre- and post-

23   anthesis applications relative to standard anthesis applications, 3 - compare the effects of anthesis and

24   pre- and post-anthesis treatments on grain yield and test weight, and 4 - determine whether wheat type,

1   as a moderator variable, affected mean responses relative to the nontreated check. These were

2   accomplished through network meta-analyses (Madden et al. 2016).

3

4   **Materials and Methods**

5   **Uniform fungicide trials**. From 1995 to 2013, more than 290 uniform fungicide trials (UFTs)

6   supported by the U.S. Wheat and Barley Scab Initiative were conducted in 17 U.S. soft and hard wheat-

7   producing states (AR, IA, IL, IN, KY, LA, MD, MI, MN, MO, MT, ND, NY, OH, SD, VA and WI)

8   (Fig. 1). The initial set of UFTs (conducted from 1995 to 2007) evaluated and compared the effects of

9   different active ingredients (a.i.), formulations, and rates of a.i. on FHB development, DON

10   accumulation, grain yield, and test weight (Hershman and Milus 2002; McMullen et al. 1999; Paul et al.

11   2007; Paul et al. 2008; Paul et al. 2010). Subsequent trials investigated the effects of different

12   combinations of demethylation inhibitor (DMI) and quinone outside inhibitor (QoI) fungicides on FHB

13   and DON when applied as a premix, a tank mix, or sequentially (Paul et al. 2018). In addition, over the

14   19 years of UFTs, several treatments were incorporated or entire trials were established to evaluate the

15   efficacy of pre- and post-anthesis applications of QoI and DMI fungicides against FHB and DON

16   (Bradley et al. 2009, 2010). Here we focus on this latter group of treatments.

17   Six UFT treatments, plus a nontreated check, were considered in this investigation. These

18   consisted of metconazole and prothioconazole + tebuconazole applied individually at Feekes growth

19   stages 10.5 (50% full head emergence), 10.5.1 (50% early anthesis), or between 5 to 7 days after 50%

20   early anthesis. All timings were based on the developmental stage of primary tillers (main stems). Prior

21   to 2008, prothioconazole + tebuconazole and metconazole were tested as experimental products under

22   various code names, and in some years, prothioconazole + tebuconazole was prepared by tank mixing

23   tebuconazole and prothioconazole (Folicur 3.6 F and Proline 480 SC, respectively; Bayer CropScience,

24   Research Triangle Park, NC) (Paul et al. 2008). For this paper, Feekes 10.5 treatments will be

25   considered as early or pre-anthesis applications and hereafter abbreviated as CE and PE for metconazole

1 and prothioconazole + tebuconazole, respectively, whereas treatments applied after 50% Feekes 10.5.1

2 will be considered as post-anthesis or late applications and abbreviated as CL and PL, respectively.

3 Treatments applied at 50% early anthesis will be considered anthesis treatments and abbreviated as CA

4 for metconazole and PA for prothioconazole + tebuconazole. Prothioconazole + tebuconazole was

5 applied at a rate of 100 g of each a.i./ha, whereas metconazole was used at 92 g a.i./ha. All treatments

6 were applied along with a non-ionic surfactant, at spray volumes ranging from 94 to 197 L ha^{-1}, using

7 either backpack or tractor-mounted sprayer equipment. The total number of trials in which each

8 treatment was evaluated for FHB index, DON, grain yield and test weight is shown in Figure 2.

9 Details pertaining to plot dimensions, cultivar characteristics, agronomic practices, inoculation,

10 and disease assessment can be found in Paul et al. (2007, 2008, 2010). In brief, plots of a susceptible

11 wheat cultivar were planted in all trials in a randomized complete block design, with 4-6 replicate

12 blocks. Plot dimensions ranged in width from 1.5 to 3 m and length from 6 to 10.7 m. To enhance spore

13 production, *F. graminearum* infection, and FHB development, plots were either planted after a *F.*

14 *graminearum*-susceptible host crop (corn or wheat), artificially infested with *F. graminearum*-colonized

15 grain spawn, or spray-inoculated with a suspension of *F. graminearum* macroconidia or a mixture of

16 macroconidia and ascospores 24-36 h after the anthesis-timed fungicide treatments were applied, and

17 then mist-irrigated during anthesis and early grain-fill. Of these approaches, the combination of

18 colonized grain spawn and mist-irrigation was the most commonly used across UFTs (Paul et al.

19 2007). FHB index was quantified as mean percentage of diseased spikelets per spike or estimated from

20 FHB incidence and disease-head severity (Paul et al. 2005; Stack and McMullen 1998). DON

21 contamination of grain, grain yield, and test weight were also quantified as previously described (Paul et

22 al. 2007, 2008). Grain yield (adjusted to 13.5% moisture) was converted to kilograms per hectare (kg ha^{-}

23 ^{1}) and test weight to kilograms per cubic meter (kg/m^3) based on a 25-kg bushel weight.

24 **Data analysis**. *Meta-analysis*. Multi-treatment or network meta-analytical models (Madden et

25 al. 2016) were fitted to the data to estimate overall measures of efficacy of each treatment against FHB

1   index (IND) and DON, to obtain overall mean estimates of yield and test weight responses to each

2   treatment, and to compare responses among early, late, and anthesis fungicide applications. Although

3   the six treatments of interest were not included in every UFT, the meta-analytical models were still

4   fitted to the entire dataset. As discussed by Madden et al. (2016), using all the data allowed us to obtain

5   direct as well as indirect evidence of treatment effects. A two-stage approach was used for all analyses.

6   In the first stage, linear mixed models were fitted in PROC GLIMMIX of SAS (SAS Inc. Cary, NC),

7   with fungicide treatment as the fixed effect and block as the random effect. Each trial, and for a given

8   trial, each response (IND, DON, YLD, and TW), was analyzed separately, and the *lsmeans* statement in

9   GLIMMIX was used to estimate least squares means for each treatment. Residual variances were also

10   estimated for each response in each trial and combined with the least squares means to construct a data

11   matrix that was used in stage two of the analyses. The matrix was completed with data for the state and

12   year in which the trial was conducted and the market class and type of the cultivar used.

13   In the second stage of the analysis, a two-way unconditional linear mixed modeling approach

14   was used to fit the network meta-analytical models (model R5 in Madden et al. [2016]) using the

15   GLIMMIX procedure of SAS, with restricted maximum likelihood as the parameter estimation method

16   (Littell et al. 2006). Overall mean log response ratios ($\overline{L}$) and mean differences ($\overline{D}$) were estimated as

17   the effect sizes to determine fungicides effects on IND and DON and grain yield and test weight,

18   respectively. Details of the meta-analytical modeling approach can be found in Madden et al. (2016) and

19   van Houwelingen et al. (2002), and complete examples of its application for estimating log response

20   ratios and mean differences as effect sizes, as well as $\hat{\sigma}^2$, are shown in Paul et al. (2008) and Paul et al.

21   (2010), respectively. In brief, for IND and DON, vectors of log-transformed treatment means were

22   constructed for each study, as well as the within-trial sampling variances for the log means in each study

23   (determined using the method described on page 1001 of Paul et al. [2008]), the latter being held fixed

24   in the network meta-analysis. Models were then fitted, and $\overline{L}$s, together with their standard errors and

25   confidence intervals, were estimated by way of *lsmestimate* and *lsmeans* (with the *diff* option)

1   statements in PROC GLIMMIX. Differences between pairs of log means are $\bar{L}$s, since the log of the

2   ratio is equal to the difference of the logs. Additionally, the between-trial variances were estimated for

3   the log ratios and mean differences ($\hat{\sigma}^2$) based on the estimated components of the between-trial

4   variance-covariance matrix (see page 1003 in Paul et al. [2008] for details).

5   For grain yield and test weight, vectors of least squares treatment means were constructed for

6   each trial, and *lsmestimate* and *lsmeans* statements in GLIMMIX were utilized to obtain the contrast

7   estimates for pairs of treatments ($\bar{D}$ values), as well as their associated standard errors and 95%

8   confidence intervals. For all measured responses (IND, DON, YLD and TW), specific contrasts were

9   constructed and corresponding measures of precision (standard errors and confidence intervals)

10   estimated to evaluate the efficacy of each of the six treatments relative to the check; the efficacy of early

11   and late applications relative to the anthesis application; and comparison of prothioconazole +

12   tebuconazole with metconazole at each application time. Estimated mean percent control ($\bar{C}$) for IND

13   and DON (reduction in the response relative to the check) and their 95% confidence intervals (CI) were

14   then estimated for all contrasts of interest from $\bar{L}$ and the upper and lower limits of the 95% CI around $\bar{L}$

15   as $[1\text{-}\exp(\bar{L})]\times100$. Because it is a back-transformation, $\bar{C}$ corresponds exactly to the median percent

16   control, but since this value is close to the mean, we refer to it as the mean. Analysis was done directly

17   on logs and not on percent control from each study because ratios (that comprise the percentage) have

18   poor statistical properties (Madden and Paul 2011).

19   *Influence of wheat type on the effect sizes*. The network meta-analytical models were refitted to

20   the data, with wheat type as a categorical moderator variable and fixed effect in the analyses. Trials

21   were categorized and coded as either a winter (W) or spring (S) wheat trial on the basis the wheat

22   market class planted. All soft red winter wheat (SRWW), hard red winter wheat (HRWW,) and soft

23   white winter wheat (SWWW) trials were coded W for winter wheat types, whereas hard red spring

24   wheat (HRSW) and Durum wheat trials were coded as S for spring wheat types. The model fitting

25   process and estimation of $\bar{L}$ and $\bar{C}$ and their standard errors and 95% CIs were as described above for the

1 analysis without the moderate variable and in Paul et al. (2008, 2010). However, instead of estimating a

2 single effect size for each contrast of interest, a pair of effect sizes was estimated, one for spring wheat

3 and another for winter wheat (the two levels of the moderator variable) by way of *lsmestimate* and

4 *lsmeans/diff* (or estimate) statements. Through *contrast* and *lsmestimate* statements in GLIMMIX, *F*

5 tests were then used to determine whether wheat type had a significant effect on the log response ratios

6 (differences in logs of FHB index or DON between pairs of treatments).

7

8 **Results**

9 **Treatment effects on FHB index (IND), deoxynivalenol (DON), grain yield and test weight.**

10 *Summary statistics*. Within-trial least squares means of IND, DON, yield, and test weight varied among

11 treatments and trials (Fig. 2). For the nontreated check, used here as a measure of baseline values for all

12 measured responses, 50% of the mean IND and DON values were between 1.5 and 21% and 0.8 and 7

13 ppm, respectively. The highest baseline IND level across 292 trials was 54%, and for DON the highest

14 baseline level across 207 trials was 32 ppm (Fig. 2A and B). Among the treatments, the overall means

15 and interquartile ranges (IQR; difference between the first and third quartiles of the data) for both

16 responses were highest for those applied prior to anthesis, PE and CE. Baseline grain yield ranged from

17 0.9 to 8 MT ha^{-1}, with 50% of the data between 2.7 and 4.4 MT ha^{-1} (Fig. 2C). The IQRs were

18 comparable among treatments, ranging from 1.6 to 1.9 MT ha^{-1}. For test weight, baseline values ranged

19 from 340 to to 830 kg/m^3, and 50% of the data were between 680 and 750 kg/m^3. The IQRs ranged from

20 34 kg/m^3 for the early metconazole (CE) treatment to 69 kg/m^3 for the two anthesis treatments, PA and

21 CA (Fig. 2D).

22 *Log response ratio and percent control for FHB index and deoxynivalenol*. All fungicide

23 treatments reduced mean IND and DON, relative to the nontreated check. For IND, the estimated

24 effective sizes ($\bar{L}$) were negative (indicating a reduction compared with the check) and significantly

25 different from zero ($P < 0.05$; Table 1 and Table 2), with the highest negative values for the anthesis

1   treatments (CA and PA), followed by the post-anthesis treatments (PL and CL), and pre-anthesis

2   treatments (CE and PE). Correspondingly, CA and PA resulted in the highest percent control, 52.2% and

3   51.5%, respectively, compared to 45.9% for CL, 41.3% for PL and less than 33% for CE and PE (Table

4   1). For both fungicides, anthesis and post-anthesis treatments resulted in significantly lower IND than

5   pre-anthesis treatments, i.e., $\bar{L}$ values were negative and significantly different from zero ($P < 0.05$) for

6   contrasts between CA and CE, CL and CE, PA and PE and PL and PE. Anthesis and post-anthesis

7   treatments reduced mean IND by 14.7 to 29.7% relative to pre-anthesis treatments. The estimated

8   effects size was also statistically significant for comparisons between CA and CL and PA and PL; CA

9   reduced IND by 11.7% relative to CL, whereas PA reduced IND by 17.4% relative to PL (Table 1).

10   Differences between pairs of prothioconazole + tebuconazole and metconazole treatments (CE vs. PE,

11   CA vs. PA, and CL vs. PL) were not statistically significant.

12   DON results were comparable to those observed for IND (Table 2), albeit with higher $\bar{L}$ and

13   smaller $\bar{C}$ values overall. The estimated effect sizes were negative and significantly different from zero

14   for all treatments ($P < 0.05$). Anthesis and post-anthesis treatments again had higher negative $\bar{L}$ values,

15   and correspondingly, resulted in greater DON reduction relative to the nontreated check ($\bar{C}$) than pre-

16   anthesis treatments. $\bar{C}$ was 42.5% and 45.4% for CA and CL, respectively, 38.2% and 37.4% for PA and

17   PL, respectively, and less than 26% for both CE and PE. Anthesis and post-anthesis treatments reduced

18   mean DON relative to pre-anthesis treatments by 27.1% and 30.7%, respectively, for metconazole, and

19   17% and 15.9%, respectively, for prothioconazole + tebuconazole. Effect sizes for contrasts between

20   anthesis and post-anthesis applications of prothioconazole + tebuconazole (PA vs. PL) or metconazole

21   (CA vs. CL) were not statistically significant. However, $\bar{L}$ values were significantly different from zero

22   for comparisons between the two fungicides for applications made at anthesis (CA vs. PA) or after (CL

23   vs. PL). CA resulted in 7% lower DON than PA, and CL in 12.8% lower DON than PL (Table 2).

24   *Mean differences for grain yield and test weight*. For both grain yield and test weight, the overall

25   mean difference relative to the nontreated check, the effect size ($\bar{D}$), was positive and significantly

1   different from zero for all treatments ($P < 0.05$, Table 3), indicating that yield and test weight were

2   higher in all treatments compared with the nontreated check. Every treatment resulted in more than a

3   330 kg ha^{-1} increase in yield, with the highest $\overline{D}$ values for the anthesis treatments, PA (431.6 kg ha^{-1})

4   and CA (398.6 kg ha^{-1}), followed by CL (358 kg ha^{-1}). $\overline{D}$ was not statistically significant for any of the

5   pairwise comparisons between metconazole treatments; yield differences were only between 20.6 and

6   61.2 kg ha^{-1}. However, for prothioconazole + tebuconazole, the anthesis treatment (PA) resulted in close

7   to 100 kg ha^{-1} higher yield than pre- or post-anthesis treatments. $\overline{D}$ was not significantly different from

8   zero ($P > 0.05$, Table 3) for comparisons between pairs of prothioconazole + tebuconazole and

9   metconazole treatments applied at the same growth stage (CE vs. PE, CA vs. PA, or CL vs. PL).

10   The test weight results followed a similar pattern to those observed for grain yield; all treatments

11   resulted in a significant increase in test weight relative to the nontreated check ($P < 0.05$; Table 3).

12    $\overline{D}$ values were higher for anthesis (PA and CA) and post-anthesis (PL and CL) treatments (16.0 to 17.7

13   kg/m^3) than for pre-anthesis treatments (approximately 11 kg/m^3 for PE and CE), and significantly

14   different from zero for comparisons between anthesis and pre-anthesis treatments and between pre- and

15   post-anthesis treatments, but not between anthesis and post-anthesis or between pairs of prothioconazole

16   + tebuconazole and metconazole treatments ($P > 0.05$, Table 3).

17   **Influence of wheat type on the efficacy of fungicide treatments against FHB index and**

18   **deoxynivalenol.** For FHB index (IND) and DON, in both spring and winter wheat, pre-anthesis,

19   anthesis, and post-anthesis applications of prothioconazole + tebuconazole and metconazole all resulted

20   in significantly lower mean levels of disease and toxin than the nontreated check. All effect sizes ($\overline{L}$)

21   were negative and significantly different from zero ($P < 0.001$, Table 4). The relative efficacy of the

22   treatments was comparable to that observed for the combined data set (without moderator variable),

23   with anthesis and post-anthesis applications resulting in higher negative $\overline{L}$ values, and consequently,

24   higher mean percent control than pre-anthesis applications in both spring and winter wheat. For IND,

1   the effect of wheat type was not statistically significant ($P > 0.05$; Table 4) for any of the tested

2   treatments; i.e., the magnitude of the effect of a treatment on IND did not depend on wheat type.

3   All treatments reduced mean DON relative to the nontreated check in both winter and spring

4   wheat (statistically significant effects sizes in Table 4), but contrary to what was observed for IND,

5   wheat type had a statistically significant effect on the efficacy of anthesis (PA and CA) and post-

6   anthesis (PL and CL) treatments, but not pre-anthesis treatments (PE and CE). Based on F tests from the

7   analyses, differences between pairs of log response ratios for comparisons of the efficacy of CA, PA,

8   CL and PL between spring and winter wheat were statistically significant ($P < 0.05$, Table 4). Estimated

9   mean effects sizes, and correspondingly, mean percent control values were significantly higher in spring

10   wheat than in winter wheat for CA, PA, CL and PL. For instance, PA and CA resulted in 10.1 and

11   10.8% lower DON than the mean for the nontreated check in spring wheat than in winter wheat,

12   respectively. Similarly, percent DON reduction relative to the check was 9.9% higher for CL and 15.6%

13   higher for PL in spring wheat than in winter wheat.

14   **Predicted efficacy against FHB index and DON in future studies**. Using effect sizes from the

15   meta-analysis ($\bar{L}$) and their respective between study variances ($\hat{\sigma}^2$), probabilities of achieving a certain

16   level of IND or DON control in a new randomly selected study, conducted in a manner similar to the

17   studies described herein, were estimated for all tested fungicide treatments (Fig. 3). For example, the

18   probability of at least a 50% control ($\bar{C} > 50\%$) of IND or DON, equivalent to the probability that $\bar{L} <$

19   -0.69, was estimated as $p_{50} = \emptyset(-0.69 - \bar{L})/\sqrt{\hat{\sigma}^2}$, where $\emptyset(\bullet)$ is the cumulative standard-normal function

20   (Paul et al. 2008, 2010). So, with an estimated effect size of -0.76 and between study variance of 0.185

21   for the effect of CA on IND in spring wheat (Table 4), p_{50} was estimated as $\emptyset(-0.69 + 0.76)/0.43 =$

22   0.56. Similarly, the $\bar{L}$ and $\hat{\sigma}^2$ values in Table 4 were used to estimate probabilities of each treatment

23   resulting in between 2 and 50% lower mean IND or DON than the mean for the nontreated check in

24   spring and winter wheat.

1   The probability of IND or DON being reduced by a certain percentage decreased for all

2   treatments as the projected percent control increased from 2 to 50% (Fig. 3). For spring wheat, anthesis

3   treatments consistently had the highest estimated probabilities for IND, followed by post-anthesis, and

4   then the pre-anthesis treatments. For instance, the estimated probability of achieving at least a 25% IND

5   reduction (p_{25}) in a new spring wheat trial was $\geq$ 0.80 for CA (0.86), CL (0.80), PA (0.82), and PL

6   (0.80), compared to 0.53 for CE and 0.61 for PE (Fig. 3A). For winter wheat, p_{25} was 0.83 for PA, 0.85

7   for CA and CL, and 0.71 for PL (Fig. 3B). Interestingly, the probability of achieving a percent control of

8   25% or greater with a pre-anthesis application varied considerably between wheat types and depended

9   on the fungicide applied. For instance, with CE, there was a 49% chance of seeing a 40% reduction in

10   IND relative to the check in winter wheat (p_{40} = 0.49) compared to only a 28% chance in spring wheat

11   (p_{40} = 0.28) (Fig. 3A and B). Conversely, with PE, the probability of achieving a 40% control of IND

12   was 0.38 in spring wheat and 0.30 in winter wheat.

13   For DON, the estimated probability of achieving a percent control of 25% or greater in a new

14   randomly selected trial was considerably higher for anthesis and post-anthesis treatments than for pre-

15   anthesis treatments, and higher in spring wheat than winter wheat (Fig. 3C and D). For example, p_{25} was

16   > 0.80 for CA (0.86), CL (0.89), PA (0.84), and PL (0.89) for spring wheat, compared to only 0.47 for

17   CE and 0.64 for PE (Fig. 3C). The corresponding probabilities for winter wheat were 0.72, 0.76, 0.66

18   and 0.62 for CA, CL, PA, and PL, respectively, and less than 0.45 for CE and PE (Fig. 3D). For both

19   wheat types, the highest probability of achieving at least a 50% reduction in DON (p_{50}) relative to the

20   nonrelated check was observed for the post-anthesis metconazole treatment (CL), but p_{50} for CL was

21   considerable higher in spring wheat, 0.53, than winter wheat, 0.33.

22

23   **Discussion**

1   For both metconazole and prothioconazole + tebuconazole, applications made before (referred to

2 here as early or pre-anthesis), at, and after (referred to as late or post-anthesis) 50% of the primary tillers

3 reached Feekes 10.5.1 (referred to as anthesis) all reduced mean FHB index (IND) and DON relative to

4 the nontreated check. This is consistent with findings from other studies investigating the effects of

5 fungicide timing on FHB and DON (D'Angelo et al. 2014; Tateishi et al. 2014; Wegulo et al. 2011).

6 Applications made before anthesis, and presumably before infection given that plots were inoculated

7 during anthesis, are considered as preventive (protective), whereas applications made after inoculation

8 (and infection) are considered curative (Chen et al. 2012; Ivec 2010). Therefore, our results demonstrate

9 that the tested fungicides have both preventive and curative effects against FHB and DON. This is not at

10 all surprising as these are properties known to be characteristic of demethylation inhibitor (DMI)

11 fungicides and have been demonstrated previously for metconazole (Tateishi et al. 2014) and related

12 active ingredients (Boyacioglu et al. 1992; Chen et al. 2012). Although all treatment programs resulted

13 in a statistically significant reduction in mean IND and DON relative to the nontreated check, the

14 magnitude of the effects in terms of percent control (used here as a measure of efficacy) was dependent

15 on the response (IND vs. DON), application timing (before, at or after anthesis), wheat type (winter vs.

16 spring wheat), and to a lesser extent, the specific fungicide (prothioconazole + tebuconazole vs.

17 metconazole).

18   For FHB index, treatments applied at anthesis (CA and PA) were the most effective, with an

19 overall (across all trials) approximate percent control of 52% for both products, followed by those

20 applied after anthesis (percent control of 46% CL and 41% for PL). Treatments applied preventively (at

21 or close to 50% full head emergence; Feekes 10.5) were the least effective, with mean percent control of

22 less than 33% for CE and PE. Based on percent control, calculated from contrasts of log-transformed

23 means across trials, anthesis treatments reduced mean IND by 29 to 30%, relative to pre-anthesis

24 treatment, and by 12 to 17% relative to post-anthesis treatments, whereas post-anthesis treatments

25 resulted in a 15 to 20% reduction in mean IND relative to pre-anthesis treatments. Like the treatments

1   applied at heading, anthesis treatments were also done preventively, but the primary difference was that

2   the latter were applied closer to the time of early anthesis, and consequently close to infection of spikes

3   on primary tillers and a few days before infection of the first spikes on secondary tillers. Heading

4   applications were made at least five days before infection of spikes on primary tillers and even earlier

5   for spikes on secondary tillers. The success of a preventative treatment is related to the amount and

6   persistence of active ingredient on the plant surface before infection (Vicent et al. 2007). Since

7   applications made well before infection were more subject to environment-, fungicide-, and host-related

8   factors known to negatively affect fungicide persistence (Anderson et al. 2014; Hunsche et al. 2016;

9   Vicent et al. 2007), the concentration of active ingredients on or in spikes treated at heading was likely

10   reduced to less effective levels, leading to reduced efficacy when compared to anthesis treatments.

11   Andersen et al. (2014) demonstrated that the half-life of tebuconazole on wheat spikes (time taken for

12   residue to decrease by 50%) was 6 to 9 days, depending on the growing season.

13   Based on results from a limited number of soft red winter wheat trials conducted under a narrow

14   range of growing condition, D'Angelo et al (2014) showed that the efficacy of post-anthesis treatments

15   also depended on how late the applications were made after approximately 50% of the spikes on primary

16   tillers reached early anthesis. They observed that curative applications closer to 50% early anthesis (2

17   days post-anthesis) were more effective in reducing IND than those applied 4 or 6 days post-anthesis.

18   Similarly, Mauler-Machnik and Suty (1997) showed that the efficacy of tebuconazole was optimum

19   when applied within a day or two of inoculation, and a longer interval before or after infection sharply

20   decreased efficiency. Although spikes on some late-developing primary tillers and secondary tillers

21   were probably between early and last anthesis when the late curative treatments were applied, most of

22   the primary spikes were probably already infected and at early grain-fill. Therefore, post-anthesis

23   treatments made between 5-7 days after 50% early anthesis of primary tillers were likely applied

24   preventively to some, but curatively to most of the spikes. The superior efficacy of anthesis treatments

25   compared to post-anthesis treatments suggests that the preventive applications made shortly before

1   infection were more effective than predominantly curative applications made five or more days after the

2   initial infection. However, when the two types of programs were compared at similar number of days

3   before or after anthesis, applications 5-7 days after infection, curatively, performed better than those

4   made the same number of days before infection, preventively. This is consistent with results from a

5   study conducted by Boyacioglu et al. (1992) to investigate the curative effects of propiconazole (Tilt 3.6

6   EC; Syngenta, Raleigh, NC) on FHB. They demonstrated that a curative application of propiconazole 2

7   days after inoculation with a spore suspension of *F. graminearum* (1×10^5 spores/ml) was more

8   effective (based on percent reduction of kernel infection relative to the nontreated check) than a

9   preventive application made 2 days before inoculation.

10   The estimated efficacy of the two fungicides evaluated was generally comparable when

11   applications were made before, at, or after anthesis. However, there was a trend towards higher efficacy

12   for metconazole over prothioconazole + tebuconazole for late applications. D'Angelo et al. (2014) also

13   alluded to numerically higher efficacy for post-anthesis applications of metconazole over post-anthesis

14   applications of prothioconazole + tebuconazole. In the current study, this difference became larger and

15   statistically significant when DON was used as a measure of treatment effect. As was the case with IND,

16   all treatments led to a significant reduction in mean DON relative to the nontreated check, but the

17   magnitude of the effect not only depended on when the fungicide was applied relative to anthesis but

18   also the active ingredient of the fungicide applied. Post-anthesis applications were just as effective as

19   anthesis applications against DON, and they were both more effective than pre-anthesis applications,

20   likely for some of the same reasons discussed above for IND. Percent control values for anthesis

21   applications were within the range of values previously reported from a meta-analysis of a smaller

22   subset of the uniform fungicide trial (UFT) data used in this study (Paul et al. 2008). However, here we

23   observed a 7% higher efficacy against DON for metconazole over prothioconazole + tebuconazole for

24   applications made at anthesis, which was slightly higher than the difference of 3-4% report by Paul el al.

25   (2008). A larger difference in efficacy (12.8%) between the two was observed for applications made at

1   5-7 days after 50% of the spikes on primary tillers were at early anthesis. Differences in terms of

2   absorption, redistribution, persistence, and curative effect are possible reasons for the observed

3   difference in estimated efficacy between metconazole and prothioconazole + tebuconazole. Similar

4   differences among DMI fungicides in terms of their curative effects have been reported for other

5   pathosystems (Holb and Schnabel 2007; Schofl and Zinkernagel 1997; Schnabel et al. 2004)**.**

6   The effect of wheat type on fungicide efficacy against IND was not statistically significant for

7   any of the fungicide x application timing combinations evaluated. Trends in winter and spring wheat in

8   terms of the magnitude and significance of treatment effects relative to the nontreated check and relative

9   to anthesis treatments were very similar to those discussed for the general situation (when efficacy was

10   estimated for the entire dataset without regard for wheat type). These results suggest that differences in

11   growing conditions between spring and winter wheat regions likely did not impact the relative

12   performance of metconazole and prothioconazole + tebuconazole applications made before, at, or, after

13   anthesis in terms of their ability to reduce the development of visual symptoms. Estimated mean percent

14   controls for treatments applied at anthesis in both wheat types were within the range of values reported

15   previously by Paul et al. (2008) from a meta-analysis of UFT data collected prior to 2006 (a subset of

16   that data was also used in this study). However, there were noticeable differences for comparisons of

17   mean percent control values between wheat types based on estimates from the current study (from the

18   analysis of larger dataset that include trials conducted between 2006 and 2013) and those reported in

19   Paul et al. (2008) for the smaller, older dataset. For instance, Paul et al. (2008), estimated the efficacy

20   for anthesis application of prothioconazole + tebuconazole and metconazole against IND and reported it

21   was significantly higher (approximately 13 and 20%, respectively) for spring wheat than winter wheat.

22   In contrast, very comparable levels of efficacy were estimated in the current study for the two wheat

23   types; 53% in spring wheat vs. 52% in winter for metconazole and 51% spring wheat vs. 52% in winter

24   wheat for prothioconazole + tebuconazole.

1   Paul et al. (2008) hypothesized that an initial difference in cultivar resistance between spring and

2   winter wheat trials was likely one of the primary reasons for the differences in fungicide efficacy they

3   observed between trials from the two wheat types. We use a similar argument to explain the lack of a

4   significant wheat type effect on the efficacy of the treatments evaluated in the current investigation

5   against IND. Although cultivars categorized as susceptible were planted in all trials, susceptible spring

6   wheat cultivars were relatively less susceptible than susceptible winter wheat cultivars prior to 2006; the

7   dataset used in Paul et al. (2008). Since that initial quantitative synthesis of data from the first eight

8   years of UFTs (1998 to 2005), twice as many new UFTs were conducted, most of which were from soft

9   red winter wheat-growing regions (Bradley, *personal communication*). During that same period,

10   considerable progress was made developing soft red winter wheat (SRWW) cultivars with FHB

11   resistance (Paul, *personal communication*), which led to old susceptible and resistant standards being

12   replaced. For instance, in Ohio, the highly susceptible SRWW cultivar Elkart was replaced by the much

13   less susceptible Hopewell in the UFTs; relative to Elkart and similar cultivars, Hopewell used to be

14   considered moderately susceptible. Such a change resulted in the dataset used in this study being

15   comprised of a higher proportion of winter wheat trials with cultivars that are relatively less susceptible

16   than those used in the first set of UFTs analyzed in Paul et al. (2008). Since fungicide efficacy against

17   IND has been shown to be greater for moderately resistant and moderately susceptible cultivars than for

18   susceptible cultivars (Willyerd et al. 2012), we hypothesize that narrowing the gap in terms of relative

19   susceptibility between winter and spring cultivars in the UFT contributed to comparable fungicide

20   efficacy between the two wheat types.

21   However, contrary to what was observed for IND, wheat type had a significant effect on the

22   efficacy of anthesis and post-anthesis applications against DON, for prothioconazole + tebuconazole or

23   metconazole. Percent DON reduction relative to the nontreated check was 10 to 16% higher with spring

24   wheat than with winter wheat. This is consistent with the results from Paul et al (2007, 2008). DON is a

25   complex trait that is not only influenced by cultivar resistance reaction and fungicide treatment effect

1   but also by several other factors not controlled by, or beyond the control of, the investigators. DON

2   levels in the grain often change after treatment application in response to weather conditions and late-

3   season infections, even if the level of visual symptoms remain relatively unchanged (Cowger et al.

4   2009; Culler et al. 2007; Del Ponte et al. 2007; Hart et al. 1984). Environmental conditions were likely

5   responsible, at least in part, for differences in the efficacy of anthesis and post-anthesis treatments

6   against DON between spring and winter wheat trials. For instance, due to cooler, wetter weather

7   conditions, the anthesis window and grain-fill periods are usually longer in winter wheat than in spring

8   wheat. This could have resulted in late infections, grain colonization, and accompanying DON

9   accumulation starting several days after the application of fungicides and thus when the fungicides were

10   less effective. These processes may have all occurred without corresponding increases in visual

11   symptom expression (Cowger and Arrellano 2010; Lacey et al. 1999), and were likely less frequent in

12   spring wheat growing regions due to much shorter anthesis and early grain-fill periods and hotter, dryer

13   post-anthesis weather conditions. Compressed anthesis and early grain-fill windows in spring wheat

14   likely result in more synchronized crop development, and consequently more spikes being treated at the

15   desired growth stage in spring wheat compared to winter wheat trials.

16   Based on a summary of 20 years (1995 to 2014) of weather data from spring and winter wheat

17   growing regions, mean daily temperature, averaged over the last 15 days before anthesis, was 3.5°C

18   warmer in spring wheat trials than in winter wheat trials, whereas mean relative humidity during the

19   same pre-anthesis window was almost identical, 69.2 and 69.1 for spring- and winter-wheat trials,

20   respectively. These data were collected from weather stations or downloaded from data archives for

21   periods and locations congruent with the UFTs (see Shah et al. [2013] for details). While these empirical

22   observations may suggest an association between local conditions and fungicide efficacy, the effect of

23   temperature on the duration of anthesis and grain-fill, and consequently, the length of the infection

24   window, is unlike the only reason for the observed differences in percent control of DON between

25   spring and winter wheat regions. Therefore, from a practical standpoint, temperature alone is unlikely to

1   be predictive of fungicide efficacy against DON. Further research would be needed to formally evaluate

2   associations between pre- and post-anthesis temperatures and the efficacy of prothioconazole +

3   tebuconazole or metconazole, as influenced by other factors such as cultivar maturity and production

4   practices that are known to affect wheat growth and development, as well as weather-, host-, pathogen-

5   related factors that affect *F. graminearum* infect, FHB development, and DON accumulation.

6   All fungicide treatments resulted in a significant increase in mean grain yield relative to the

7   nontreated check. The magnitude of the response was similar among metconazole treatments, but higher

8   for the anthesis application compared to pre- or post-anthesis applications for prothioconazole +

9   tebuconazole. The superior efficacy of the anthesis and post-anthesis metconazole treatments over the

10   pre-anthesis treatments against FHB visual symptom development did not translate into significantly

11   higher yield gains. This is not uncommon for fungicide trials, as yield response is not only a function of

12   the diseases being targeted. In fact, for prothioconazole + tebuconazole, the yield response was likely

13   due not only to superior FHB control but also to the control of other diseases during the critical grain-fill

14   period, as this fungicide is known to be very effective against a number of foliar diseases (Salgado et al.

15   2017; Willyerd et al. 2015). Keeping the spikes and upper leaves disease-free, or at a reduced disease

16   pressure, is important for grain yield and quality (Ruske et al. 2003; Simpson 1968). Although yield is

17   very important, grain quality in terms of DON reduction and test weight increase is often just as or even

18   more important when managing FHB. Results from economic analyses of data from FHB management

19   trials showed that even if there is a yield increase in response to a given management program, if that

20   program does not reduce DON below, and increase test weight above, critical grain marketing

21   thresholds, economic losses may still occur as a result of price discounts (Cowger et al. 2016; Salgado et

22   al. 2014). In this study, test weight increases were associated with efficacy against IND as the four

23   treatments (PA, PL, CA, and CL) that resulted in the highest mean percent control of IND also resulted

24   in the highest mean increases in test weight. This is likely because there is often a significant negative

25   relationship between IND and test weight (Salgado et al 2015), as FHB development is commonly

1 associated with damage to kernels (shriveled and lightweight) which directly affects test weight (a

2 measure of grain weight for unit volume).

3 In this study we not only estimated the overall efficacy of prothioconazole + tebuconazole and

4 metconazole, two of the most effective and widely used fungicide for FHB management, we also

5 generated information useful for making future application decisions in terms when choosing between

6 the two products and among the three tested application times. We present evidence confirming the

7 importance of anthesis as an appropriate target growth stage for applying fungicides to manage FHB.

8 Since both prothioconazole + tebuconazole and metconazole are rainfast within the first hour after

9 treatment application (Anderson et al 2014; Mills et al. 2017) producers should strive to apply

10 treatments at anthesis even if rain is in the forecast. However, if adverse field conditions or other factors

11 prevent applications from being made at anthesis, the next best option according to our results would be

12 to apply treatments within a 5-7 day window after rather than in the 5-7 days before anthesis. Missing

13 the anthesis window therefore should not be a justification for not treating fields when the risk of FHB is

14 high. Based on estimated probabilities of percent control in future studies (Fig. 3), producers are more

15 likely to see the highest levels of FHB and DON control if they choose to apply treatments at or shortly

16 after anthesis than at heading, which is a few days before anthesis. Late applications were just as

17 effective as anthesis applications at reducing DON, and if a late application has to be made, metconazole

18 would be a better choice of fungicide. This was the case for both wheat types, although fungicide

19 efficacy for DON was higher with spring than with winter wheat.

20

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22

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1 **Table 1.** Overall mean effect sizes (log response ratios), percent control and corresponding statistics for the effect of fungicide treatments on
2 Fusarium head blight index (IND; mean percentage of diseased spikelets per spike) in wheat

Contrast[a]	Effect size[b]					Mean percent control[c]		
	$\bar{L}$	$se(\bar{L})$	CI_L	CI_U	P	$\bar{C}$	CI_L	CI_U
Between treatment and the nontreated check								
CE vs. CK	-0.40	0.056	-0.51	-0.29	<0.001	32.78	25.03	39.72
CA vs. CK	-0.74	0.038	-0.81	-0.67	<0.001	52.23	48.57	55.64
CL vs. CK	-0.61	0.048	-0.71	-0.52	<0.001	45.93	40.64	50.75
PE vs. CK	-0.37	0.053	-0.48	-0.27	<0.001	31.00	23.43	37.83
PA vs. CK	-0.72	0.039	-0.80	-0.65	<0.001	51.51	47.64	55.09
PL vs. CK	-0.53	0.044	-0.62	-0.45	<0.001	41.29	36.05	46.09
Between application timings								
CA vs. CE	-0.34	0.053	-0.45	-0.24	<0.001	28.95	21.09	36.02
CA vs. CL	-0.12	0.047	-0.22	-0.03	0.008	11.66	3.16	19.42
CL vs. CE	-0.22	0.059	-0.33	-0.10	<0.001	19.57	9.61	28.42
PA vs. PE	-0.35	0.054	-0.46	-0.25	<0.001	29.72	21.92	36.74
PA vs. PL	-0.19	0.045	-0.28	-0.10	<0.001	17.41	9.80	24.38
PL vs. PE	-0.16	0.059	-0.28	-0.05	0.006	14.91	4.54	24.14
Between fungicides								
CE vs. PE	-0.03	0.063	-0.15	0.10	0.678	2.57	-10.16	13.83
CA vs. PA	-0.02	0.031	-0.08	0.05	0.630	1.50	-4.73	7.35
CL vs. PL	-0.08	0.051	-0.18	0.02	0.107	7.91	-1.78	16.68

3 [a]Nontreated check (CK), metconazole applied before (CE), at (CA), or after (CL) 50% early anthesis (Feekes 10.5.1) and prothioconazole +
4 tebuconazole applied before (PE), at (PA), or after (PL) early anthesis.
5 [b]$\bar{L}$ = mean log of the response ratio between pairs of treatments, $se(\bar{L})$ = standard error of $\bar{L}$, P = significance level, and CI_U and CI_L = upper
6 and lower limits of the 95% confidence interval around $\bar{L}$.
7 [c]$\bar{C}$ = mean percent control was estimated from $\bar{L}$ as $\bar{C} = [1-exp(\bar{L})] \cdot 100$; CI_U and CI_L = upper and lower limits of the 95% confidence
8 interval around $\bar{C}$.
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1 **Table 2.** Overall mean effect size (log response ratio), percent control and corresponding statistics for the effect of fungicide treatments on
2 deoxynivalenol (DON) content of wheat grain

Contrast[a]	Effect size[b]					Mean percent control[c]		
	$\bar{L}$	se($\bar{L}$)	CI_L	CI_U	P	$\bar{C}$	CI_L	CI_U
Between treatment and the nontreated check								
CE vs. CK	-0.24	0.037	-0.31	-0.17	<0.001	21.17	15.32	26.62
CA vs. CK	-0.55	0.034	-0.62	-0.49	<0.001	42.52	38.52	46.26
CL vs. CK	-0.61	0.044	-0.69	-0.52	<0.001	45.39	40.50	49.88
PE vs. CK	-0.30	0.036	-0.37	-0.22	<0.001	25.56	20.07	30.67
PA vs. CK	-0.48	0.031	-0.54	-0.42	<0.001	38.19	34.34	41.82
PL vs. CK	-0.47	0.038	-0.54	-0.39	<0.001	37.40	32.56	41.90
Between application timings								
CA vs. CE	-0.32	0.040	-0.39	-0.24	<0.001	27.08	21.12	32.59
CA vs. CL	0.05	0.038	-0.02	0.13	0.175	-5.26	-13.36	2.25
CL vs. CE	-0.37	0.047	-0.46	-0.28	<0.001	30.72	24.04	36.82
PA vs. PE	-0.19	0.040	-0.26	-0.11	<0.001	16.97	10.19	23.23
PA vs. PL	-0.01	0.034	-0.08	0.05	0.711	1.26	-5.61	7.69
PL vs. PE	-0.17	0.044	-0.26	-0.09	<0.001	15.91	8.24	22.93
Between fungicides								
CE vs. PE	0.06	0.044	-0.03	0.14	0.189	-5.90	-15.36	2.78
CA vs. PA	-0.07	0.025	-0.12	-0.02	0.004	7.00	2.26	11.50
CL vs. PL	-0.14	0.041	-0.22	-0.06	0.001	12.76	5.46	19.50

3 [a]Nontreated check (CK), metconazole applied before (CE), at (CA), or after (CL) 50% early anthesis (Feekes 10.5.1) and prothioconazole +
4 tebuconazole applied before (PE), at (PA), or after (PL) early anthesis.
5 [b]$\bar{L}$ = mean log of the response ratio between pairs of treatments, se($\bar{L}$) = standard error of $\bar{L}$, P = significance level, and CI_U and CI_L = upper
6 and lower limits of the 95% confidence interval around $\bar{L}$.
7 [c]$\bar{C}$ = mean percent control was estimated from $\bar{L}$ as $\bar{C}$ = [1-$exp(\bar{L})$]•100; CI_U and CI_L = upper and lower limits of the 95% confidence
8 interval around $\bar{C}$.

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1 **Table 3.** Overall mean effect sizes (difference in grain yield and test weight) and corresponding statistics for the effect of fungicide
2 treatments for Fusarium head blight management on grain yield and test weight

Contrast[a]	Grain yield[b]					Test weight[b]				
	$\bar{D}$	se($\bar{D}$)	CI_L	CI_U	P	$\bar{D}$	se($\bar{D}$)	CI_L	CI_U	P
Between treatment and the nontreated check										
CE vs. CK	337.41	40.21	258.55	416.28	<0.001	11.21	1.83	7.61	14.81	<0.001
CA vs. CK	398.62	22.05	355.38	441.87	<0.001	16.18	1.65	12.94	19.42	<0.001
CL vs. CK	358.03	28.06	303.00	413.06	<0.001	17.76	2.14	13.56	21.97	<0.001
PE vs. CK	334.99	37.92	260.62	409.37	<0.001	10.98	1.74	7.57	14.39	<0.001
PA vs. CK	431.58	22.39	387.66	475.50	<0.001	16.00	1.23	13.59	18.41	<0.001
PL vs. CK	333.97	28.34	278.38	389.55	<0.001	17.23	2.00	13.31	21.14	<0.001
Between application timings										
CA vs. CE	61.21	37.70	-12.73	135.15	0.105	4.97	1.58	1.86	8.08	0.002
CA vs. CL	40.59	29.65	-17.57	98.76	0.171	-1.58	1.38	-4.28	1.11	0.249
CL vs. CE	20.62	43.26	-64.24	105.48	0.634	6.56	1.81	3.00	10.11	<0.001
PA vs. PE	96.58	37.31	23.40	169.77	0.010	5.02	1.51	2.06	7.97	0.001
PA vs. PL	97.61	30.86	37.07	158.15	0.002	-1.23	1.42	-4.02	1.56	0.386
PL vs. PE	-1.03	41.49	-82.41	80.36	0.980	6.25	1.82	2.68	9.81	0.001
Between fungicides										
CE vs. PE	2.42	46.46	-88.72	93.56	0.959	0.23	1.84	-3.38	3.84	0.901
CA vs. PA	-32.95	18.88	-69.98	4.07	0.081	0.18	1.00	-1.77	2.14	0.853
CL vs. PL	24.06	32.07	-38.83	86.96	0.453	0.54	1.41	-2.24	3.31	0.704

3 [a]Nontreated check (CK), metconazole applied before (CE), at (CA) or after (CL) 50% early anthesis (Feekes 10.5.1) and prothioconazole +
4 tebuconazole applied before (PE), at (PA), or after (PL) early anthesis.
5 [b]$\bar{D}$ = mean difference in yield between pairs of treatments, se($\bar{D}$) = standard error of $\bar{D}$, and CI_U and CI_L = upper and lower limits of the 95%
6 confidence interval around $\bar{D}$, and P = significance level.

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1 **Table 4.** Overall mean log response ratios (effect sizes), percent control, and corresponding statistics for the influence of wheat type on the
2 effects of fungicides on Fusarium head blight index (IND; mean percentage of diseased spikelets per spike) and deoxynivalenol (DON) in
3 wheat

Response[a]	Contrast[b]	Type[c]	Effect size[d]						Mean percent control[e]		
			$\bar{L}$	se($\bar{L}$)	CI_L	CI_U	$\hat{\sigma}^2$	P	$\bar{C}$	CI_L	CI_U
IND	CE vs. CK	Spring	-0.31**	0.074	-0.46	-0.16	0.119	0.072	26.61	15.08	36.58
		Winter	-0.50**	0.075	-0.65	-0.35			39.33	29.71	47.63
	CA vs. CK	Spring	-0.76**	0.054	-0.87	-0.66	0.185	0.631	53.32	48.15	57.98
		Winter	-0.73**	0.051	-0.83	-0.63			51.63	46.51	56.25
	CL vs. CK	Spring	-0.59**	0.067	-0.72	-0.46	0.126	0.502	44.60	36.83	51.40
		Winter	-0.65**	0.067	-0.78	-0.52			48.00	40.74	54.37
	PE vs. CK	Spring	-0.39**	0.076	-0.54	-0.24	0.149	0.458	32.49	21.59	41.87
		Winter	-0.31**	0.075	-0.46	-0.17			26.90	15.23	36.96
	PA vs. CK	Spring	-0.72**	0.056	-0.83	-0.61	0.216	0.819	51.26	45.59	56.35
		Winter	-0.74**	0.052	-0.84	-0.63			52.11	46.94	56.79
	PL vs. CK	Spring	-0.58**	0.064	-0.71	-0.45	0.121	0.279	44.02	36.51	50.64
		Winter	-0.48**	0.062	-0.60	-0.36			38.36	30.45	45.37
DON	CE vs. CK	Spring	-0.27**	0.057	-0.39	-0.16	0.036	0.409	23.95	15.00	31.95
		Winter	-0.21**	0.048	-0.31	-0.12			19.16	11.25	26.35
	CA vs. CK	Spring	-0.67**	0.056	-0.78	-0.56	0.120	0.012	48.69	42.68	54.07
		Winter	-0.49**	0.043	-0.57	-0.40			38.61	33.17	43.61
	CL vs. CK	Spring	-0.72**	0.071	-0.86	-0.58	0.125	0.040	51.37	44.13	57.67
		Winter	-0.54**	0.056	-0.65	-0.43			41.47	34.65	47.57
	PE vs. CK	Spring	-0.34**	0.057	-0.46	-0.23	0.026	0.298	29.13	20.74	36.64
		Winter	-0.27**	0.047	-0.36	-0.17			23.44	15.96	30.25
	PA vs. CK	Spring	-0.59**	0.051	-0.69	-0.49	0.091	0.005	44.67	38.83	49.96
		Winter	-0.41**	0.038	-0.49	-0.34			33.87	28.79	38.59
	PL vs. CK	Spring	-0.63**	0.062	-0.76	-0.51	0.081	0.001	46.95	40.08	53.03
		Winter	-0.38**	0.049	-0.47	-0.28			31.35	24.48	37.59

4 [a]IND = Fusarium head blight index (mean percentage of diseased spikelets per spike) and DON = deoxynivalenol content of harvested grain.
5 [b]Nontreated check (CK), metconazole applied before (CE), at (CA), or after (CL) 50% early anthesis (Feekes 10.5.1) and prothioconazole + tebuconazole applied
6 before (PE), at (PA), or after (PL) early anthesis.
7 [c]Data obtained from winter wheat and spring wheat studies.
8 [d]$\bar{L}$ = mean log of the response ratio between pairs of treatments means, se($\bar{L}$) = standard error of $\bar{L}$, CI_L and CI_U = lower and upper limits of the 95% confidence
9 interval around $\bar{L}$, $\hat{\sigma}^2$ = between study variance, P = significance level for wheat type, and ** = statistical significance of $\bar{L}$ ($P < 0.001$).
10 [e]$\bar{C}$ = mean percent control estimated as $\bar{C}$ = [1-$exp(\bar{L})$]•100; CI_L and CI_U = lower and upper limits of the 95% confidence interval around $\bar{C}$.

1 **Fig. 1.** Map showing states from which data were collected for this study. Shades of gray indicate
2 soft red winter (SRWW), soft white winter (SWWW), hard red winter (HRWW), and hard red spring
3 (HRSW) wheat market classes. Numbers are the number of uniform fungicide trials included from
4 each of the participating states.
5
6 **Fig. 2.** Box plots summarizing the distribution of **A***,* Fusarium head blight index (mean percentage of
7 diseased spikelets per spike), **B**, deoxynivalenol content of harvested wheat grain, and **C**, grain yield
8 and **D**, test weight for different fungicide treatments plus an nontreated check (CK). Treatments were
9 prothioconazole + tebuconazole applied before (PE), at (PA), and after (PL) 50% early anthesis
10 (Feekes 10.5.1) and metconazole before (CE), at (CA) and after (CL) early anthesis. Broken and
11 solid lines within each box represent means and medians, respectively, while the top and bottom lines
12 of the box represent the 75th and 25th percentiles of the data, respectively. Vertical bars extending
13 beyond the boxes represent the 10th and 90th percentiles, while circles represent outliers.
14
15 **Fig. 3.** Estimated probabilities of observing percent controls ranging from at least 2% to at least 50%
16 for **A** and **B**, Fusarium head blight index (FHB) and **C** and **D**, deoxynivalenol contamination of
17 harvested grain relative to a nontreated check following the application of different fungicide
18 treatments to FHB affected plots in a new randomly selected spring or winter wheat trial. Treatments
19 consisted of prothioconazole + tebuconazole applied before (PE), at (PA), or after (PL) 50% early
20 anthesis (Feekes 10.5.1) and metconazole before (CE), at (CA), or after (CL) early anthesis.
21 Probability values were calculated using the mean log response ratios and their between-study
22 variances in Tables 4 estimated through network meta-analysis. Horizontal reference lines show
23 probabilities of 0.25, 0.50, and 0.75 and vertical reference lines show 25% control.
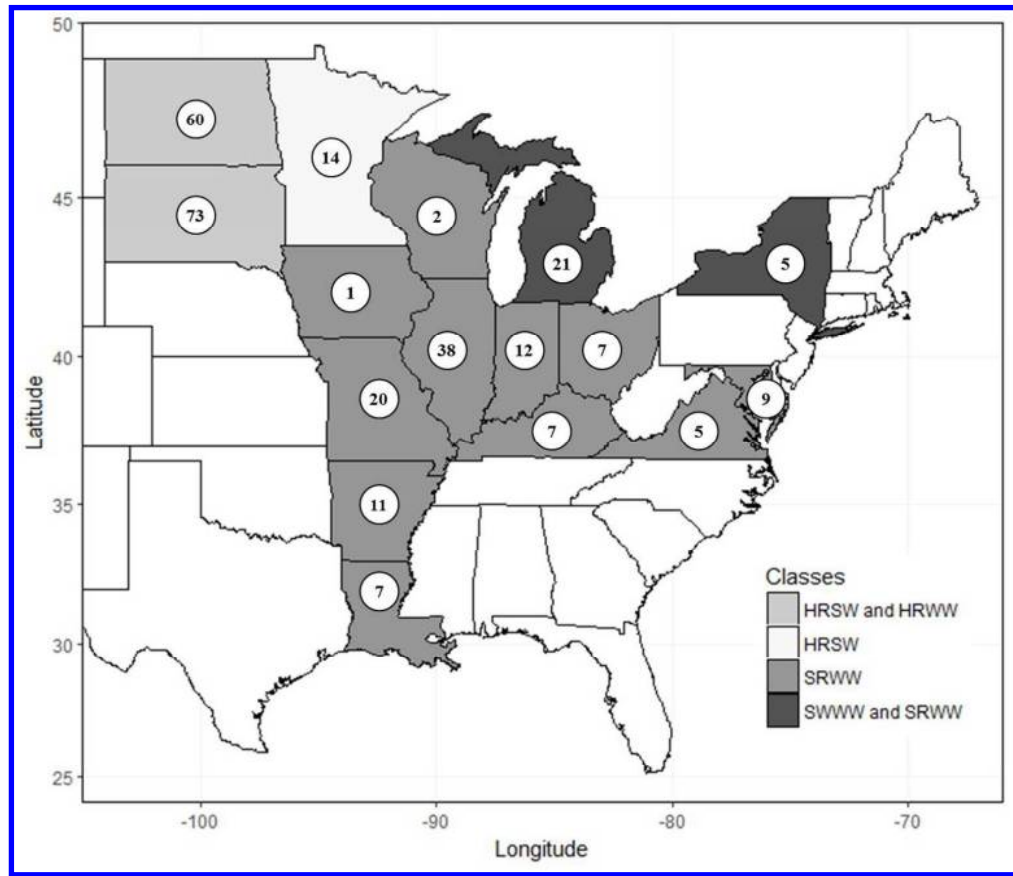24

Fig. 1. Map showing states from which data were collected for this study. Shades of gray indicate soft red winter (SRWW), soft white winter (SWWW), hard red winter (HRWW), and hard red spring (HRSW) wheat market classes. Numbers are the number of uniform fungicide trials included from each of the participating states.
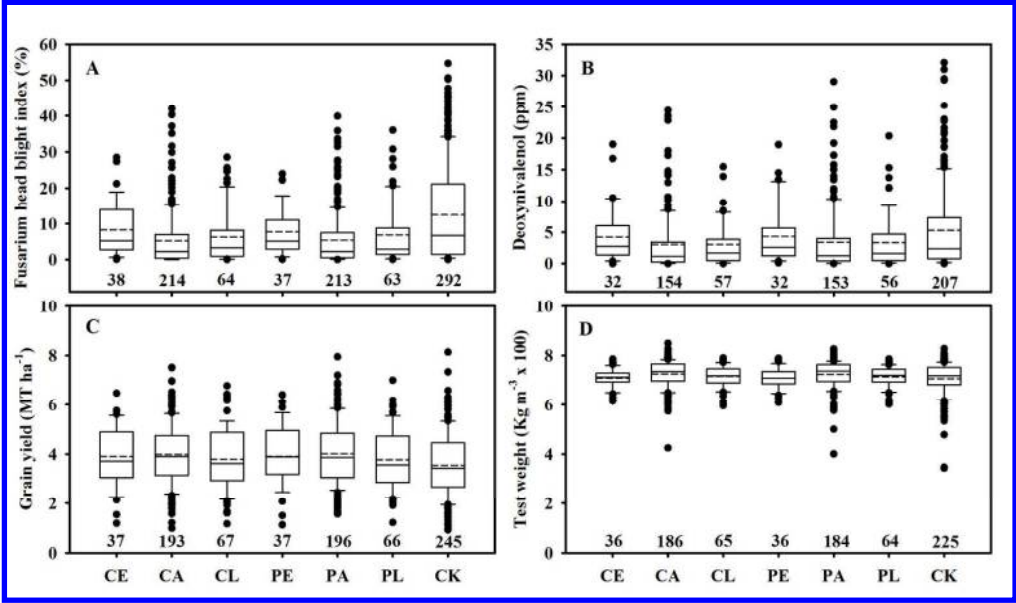
219x188mm (150 x 150 DPI)

Fig. 2. Box plots summarizing the distribution of A, Fusarium head blight index (mean percentage of diseased spikelets per spike), B, deoxynivalenol content of harvested wheat grain, and C, grain yield and D, test weight for different fungicide treatments plus an nontreated check (CK). Treatments were prothioconazole + tebuconazole applied before (PE), at (PA), and after (PL) 50% early anthesis (Feekes 10.5.1) and metconazole before (CE), at (CA) and after (CL) early anthesis. Broken and solid lines within each box represent means and medians, respectively, while the top and bottom lines of the box represent the 75th and 25th percentiles of the data, respectively. Vertical bars extending beyond the boxes represent the 10th and 90th percentiles, while circles represent outliers.
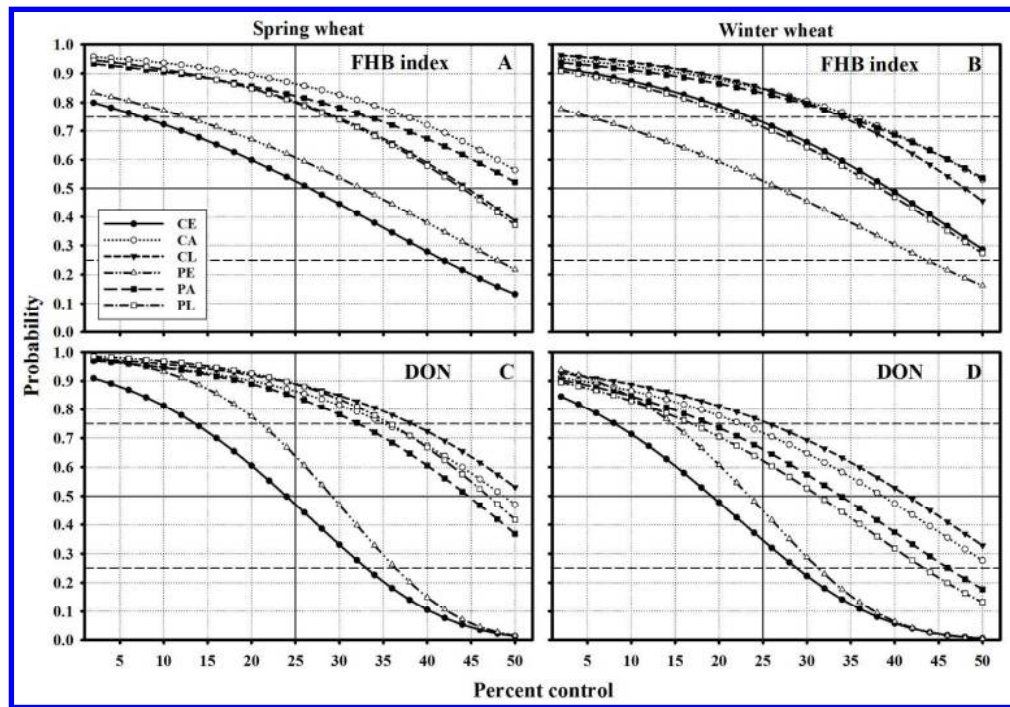
358x211mm (150 x 150 DPI)

Fig. 3. Estimated probabilities of observing percent controls ranging from at least 2% to at least 50% for A and B, Fusarium head blight index (FHB) and C and D, deoxynivalenol contamination of harvested grain relative to a nontreated check following the application of different fungicide treatments to FHB affected plots in a new randomly selected spring or winter wheat trial. Treatments consisted of prothioconazole + tebuconazole applied before (PE), at (PA), or after (PL) 50% early anthesis (Feekes 10.5.1) and metconazole before (CE), at (CA), or after (CL) early anthesis. Probability values were calculated using the mean log response ratios and their between-study variances in Tables 4 estimated through network meta-analysis. Horizontal reference lines show probabilities of 0.25, 0.50, and 0.75 and vertical reference lines show 25% control.

396x273mm (150 x 150 DPI)