



Impact of *Fusarium graminearum* inoculum availability and fungicide application timing on Fusarium head blight in wheat



Anna N. Freije, Kiersten A. Wise*

Purdue University, 915 West State Street, West Lafayette, IN 47907, USA

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ABSTRACT

Fusarium head blight (FHB) of wheat (caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch) is considered one of the most economically important diseases on wheat in the United States. Currently, farmers rely heavily on fungicides applied at early anthesis or Feekes Growth Stage (FGS) 10.5.1 to protect their crop from this disease. Field trials were conducted at the Agronomy Center for Research and Education in West Lafayette, IN during the 2012–2013 and 2013–2014 growing seasons to determine the impact of post-anthesis fungicide timing in conjunction with initial infection by *F. graminearum* on development of FHB and deoxynivalenol (DON) in soft red winter wheat. To achieve this, each experimental plot within a treatment was inoculated and received a fungicide application on the same day beginning at FGS 10.5.1 (anthesis), and continuing each day for anthesis +1, 3, 5, 7, 9, and 11 days. The fungicide prothioconazole + tebuconazole was applied at 475 mL/ha, and experimental plots were inoculated with macroconidia of *F. graminearum* on the same day as each fungicide application. Results indicate that fungicide applications made up to 11 days post-anthesis may be useful in reducing FHB and DON in wheat when inoculum becomes present near the time of application.

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1. Introduction

Fusarium graminearum Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch] is the primary causal agent of Fusarium head blight (FHB) of wheat (*Triticum aestivum* L. ssp. *aestivum*) in the United States (Goswami and Kistler, 2004). This fungus infects wheat heads during anthesis, causing salmon to white colored “tombstone” kernels to form in lieu of healthy grain (Sutton, 1982). The fungus also produces several mycotoxins, including deoxynivalenol (DON), which is known to inhibit protein synthesis in eukaryotes, making it harmful to humans and other mammals (O'Donnell et al., 2000). Although FHB began as a sporadic problem in the U.S. since the mid 1920's, it re-emerged as a disease of great economic importance after a series of epidemics in the mid 1990's (McMullen et al., 1997, 2012). The pathogen is also able to infect corn (*Zea mays* L.), another important crop in the Midwestern U.S., causing the disease Gibberella ear rot (Sutton, 1982). Currently, FHB is considered the disease of greatest concern to wheat cultivation in the U.S. (Bockus et al., 2010).

No single management practice will completely suppress FHB. Typical integrated pest management (IPM) strategies for FHB include planting wheat after soybean instead of after corn, using moderately resistant wheat cultivars, and applying fungicide at beginning anthesis (Mesterházy, 1995; Willyerd et al., 2011). The most effective fungicides currently labeled for use against *F. graminearum* on wheat are the demethylase inhibitor (DMI) triazoles, prothioconazole and tebuconazole, trade name Prosaro (Bayer CropScience LP, Research Triangle Park, NC), and metconazole, trade name Caramba (BASF, Research Triangle Park, NC; FRAC, 2011; Paul et al., 2008; Wise, 2014). All of the active ingredients in these fungicides are sterol biosynthesis inhibitors (SBI) and are members of fungicide resistance action committee (FRAC) group G1: SBI class 1: DMI fungicides (FRAC code 3; FRAC, 2011). Both products are broad-spectrum fungicides and are also used to control foliar diseases in wheat (Wise, 2014). The DMI triazole fungicides inhibit sterol biosynthesis in fungal membranes by inhibiting the action of enzyme C14-demethylase on C-14 in lanosterol. This is a necessary step in the biosynthesis of ergosterol, and its inhibition leads to the buildup of fatty acids and ergosterol precursors in the fungal cells, resulting in abnormal growth patterns and inhibition of growth (Köller, 1992; Schnabel and Jones, 2001; Siegel, 1981). The DMI triazole fungicides are also partially systemic, meaning they can

* Corresponding author. Dept. of Botany and Plant Pathology, Purdue University, 915 West State Street, West Lafayette, IN 47907, USA.

E-mail address: kawise@purdue.edu (K.A. Wise).

penetrate the plant and move within its tissue, but they are unable to enter the xylem (Mueller and Bradley, 2008; Siegel, 1981).

Proper application timing and techniques are essential for optimum fungicide efficacy. Current recommendations state that fungicide should be applied at early anthesis, or Feekes Growth Stage (FGS) 10.5.1, the time at which 50% of the primary tillers in a field have 50% of their anthers extruding (Large, 1954). In winter wheat there are several limitations to meeting this optimal application timing. Winter wheat may produce many tillers and therefore beginning anthesis on different heads may be spread over several days. Because of this, the flowering period of a single plant can extend up to two weeks. Spraying fungicide at beginning anthesis of the primary tiller will not coincide with beginning anthesis for the secondary tillers. Weather has a great influence on spore production and infection by *F. graminearum*. When flowering of individual heads within a field is staggered over a week or more, some heads will be more vulnerable to infection than others. Likewise, if fungicide timing is truly critical, the variability of flowering wheat heads within a field will impact the efficacy of a fungicide application. Rain can also pose an obstacle to spraying at precisely FGS 10.5.1 due to the inability of spray equipment to enter a wet field. Several studies have also determined that the optimum application timing for FHB suppression and DON reduction may be different (Yoshida et al., 2012; Yoshida and Nakajima, 2010). Finally, it is important that fungicide applications do not violate the fungicide pre-harvest interval, the amount of time that must pass between the final fungicide application and harvest. If fungicide is applied after the optimum timing and weather conditions favor a prompt harvest, it is possible that the 30-day pre-harvest interval for commonly applied fungicides will not be met.

Several studies have demonstrated that fungicide applications can reduce FHB and DON levels when applied up to 6 days past FGS 10.5.1 and that DON may be reduced by applications made up to 20 days after anthesis (DAA) (D'Angelo et al., 2014; Hart et al., 1984; Yoshida et al., 2012). However, all of these studies have focused on the effect of post-anthesis fungicide applications when inoculum became available at FGS 10.5.1. Since inoculum availability is primarily influenced by environmental conditions, it is likely that infection does not always occur precisely at FGS 10.5.1. Also, because anthesis can last approximately 2 weeks, it is important to determine the efficacy of fungicide use during this entire period of host susceptibility. Therefore, the objective of this study was to determine the impact of fungicide timing, in conjunction with initial infection by *F. graminearum*, on FHB and DON up to 11 days post-anthesis in soft red winter wheat.

2. Materials and methods

Field studies were conducted in two field seasons, 2012–2013 and 2013–2014, at Purdue's Agronomy Center for Research and Education (ACRE) in West Lafayette, Indiana.

2.1. 2013 Field experiment

Plots were established on October 12, 2012 with soft red winter wheat cultivar P25R47, which is moderately susceptible to FHB. Seed was drilled at a seeding rate of 5.0×10^6 seeds/ha using a Great Plains drill into soil that had been disked and field cultivated after a crop of corn. Fertilizer in the form of diammonium phosphate (DAP) was applied at 100.8 kg/ha on September 19, 2012, followed by an application of potash at 336 kg/ha on September 25 and an application of urea at 224 kg/ha on March 21, 2013. Weeds were controlled by hand prior to anthesis.

The experimental design was a randomized complete block with a 2×7 factorial arrangement, and was replicated four times within

the experiment. Factorial level one refers to the presence or absence of fungicide given an inoculation with *F. graminearum*. Level two refers to the timing, or day that fungicide and inoculum were applied relative to the beginning of anthesis (FGS 10.5.1). Anthesis was defined as the first day that 50% of the primary tillers across the field were extruding 50% of their anthers. The application (both fungicide and inoculum) occurring at anthesis was given a designation of day 0. Applications occurring after anthesis were designated as the number of days after anthesis (DAA), with six applications occurring at 1, 3, 5, 7, 9, or 11 DAA. In 2013, anthesis occurred on May 25. Each plot was designated as an experimental unit and one of the seven application timings (0–11 DAA) was randomly assigned to each experimental unit within a replicate. Each plot received a maximum of one fungicide application corresponding to the randomly assigned day after anthesis. The inoculated, non-fungicide treated plots served as controls within each application time.

Experimental plots were 2.1 m wide and 6.1 m long with a 1.5 m wide alley between each plot. Border plots of the same size were established between experimental plots to prevent the effects of inoculum and/or fungicide drift during treatment applications. Border plots were planted with cultivar INW0803 at a seeding rate of 3.4×10^6 seeds/ha.

2.2. 2014 Field experiment

Plots were established on October 15, 2013 with soft red winter wheat cultivar P25R47 at a seeding rate of 3.4×10^6 seeds/ha using a Great Plains Drill. The previous crop was corn. The field was disked four times prior to planting. Fertilizer, in the form of DAP (at a rate of 100.8 kg/ha) and nitrogen (at a rate of 107.3 kg/ha), was applied on September 2, 2013 and March 28, 2014 respectively. Weeds were controlled by hand prior to anthesis. Due to a harsh winter that led to winter kill of wheat plants, the healthiest 21 plots in each replication, from a total of 30, were selected for use in the experiment prior to treatment randomization.

The experimental design in 2014 consisted of a randomized complete block with a 3×7 factorial arrangement and was replicated four times within the experiment. Factorial level one refers to the presence or absence of fungicide given an inoculation with *F. graminearum*. A naturally infected, non-fungicide treated control was added in 2014, which served as a means of evaluating the baseline level of disease in naturally infected plots alongside inoculated plots. Level two refers to the timing, or day that fungicides and inoculum were applied relative to the beginning of anthesis (FGS 10.5.1). Anthesis was defined as above, and the application (both fungicide and inoculum) occurring at anthesis was given a designation of day 0. Treatments occurred on 1, 3, 5, 8, 9, or 11 days after anthesis (DAA). In 2014, FGS 10.5.1 occurred on May 28. Treatment applications scheduled for 7 DAA were moved to 8 DAA due to rain. Experimental and border plots were established as described for the 2013 experiment (2.1).

2.3. Inoculum preparation

Macroconidia inoculum of *F. graminearum* was prepared in the laboratory prior to field inoculation. Isolates used had been stored on corn kernels at -80°C storage prior to use. We used a mix of isolates collected in Indiana each year to simulate typical local inoculum pressure. In 2013 the isolates 09INDecaturF3S1, 09INDecaturF1S1, and 10INSWS2U112 were selected for inoculation. In 2014, isolates 09DecaturF3S1, 10INSWS2U112 and 13INHunt600NPH5 were used in the field trial. Each isolate was screened for virulence on wheat in a greenhouse prior to use in these trials. Isolates were grown on full strength potato dextrose

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