Assessing the Curative and Protective Impacts of Select Fungicides for Control of Powdery Mildew of Wheat

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Abstract

Powdery mildew, caused by the obligate fungal pathogen *Blumeria graminis*, has been increasing in incidence and severity on wheat in the Chesapeake Bay region of the mid-Atlantic United States. Although fungicides are used for in-season management of powdery mildew, no studies to date have assessed the ability of these products to control disease after pathogen arrival/infection (curative activity) and the duration of disease control provided after fungicide application (protective activity) under controlled conditions. Five commercially available fungicide products, including Caramba, Stratego YLD, Priaxor, Prosaro, and Triapro, were applied at either 3 or 5 days after inoculation with *B. graminis* spores to assess curative activity. In a separate study, protective activity for these fungicides was assessed by applying fungicides and inoculating with *B. graminis* spores the same day and 21 and 42 days after

fungicide application. All fungicides reduced powdery mildew severity on foliage compared with nontreated controls. Priaxor provided 11 to 18% less activity than other fungicides when applied curatively. All products provided protective control up to 42 days after inoculation, with Stratego YLD and Priaxor providing the greatest level of control at 68 and 56%, respectively. Our data indicate that different fungicides can have subtle differences in overall efficacy profiles that may translate to improved control or an extended control window in some situations, but all tested products provide very good to excellent control of powdery mildew on wheat.

Keywords: chemical, cereals and grains, disease management, field crops, fungi, techniques

Powdery mildew, caused by the fungus *Blumeria graminis* f. sp. *tritici* (syn. *Erysiphe graminis*), is a foliar disease that is frequently encountered in wheat production systems (Cowger et al. 2012). Like all powdery mildews, *B. graminis* (Bg) is an obligate biotrophic pathogen, requiring a living host to grow and complete its life cycle. Bg is considered a cool season pathogen, favored by temperatures between 15 and 22°C and relative humidity between 85 and 100% (Stromberg 2010). Under favorable conditions, new conidia can be produced 7 to 10 days after initial infection of tissues, resulting in severe epidemics because multiple disease cycles will occur with prolonged optimal conditions. Severe infections rarely result in plant death but can significantly reduce yields (Murray and Brennan 2009).

Soft red winter wheat is a significant crop in the mid-Atlantic United States as a result of the surrounding flour, feed, and burgeoning microbrewery and distillation industries. In 2018, 176,038 ha of soft red winter wheat were planted in Delaware and Maryland, with production valued at \$31,816,000 USD (U.S. Department of Agriculture National Agricultural Statistics Service 2019). Powdery mildew is an economically important disease of wheat in this region, which has moderate temperatures and high relative humidity during the growing season owing to the Chesapeake Bay and Atlantic Ocean (Sylvester and Kleczewski 2018). Yield losses caused by powdery mildew can exceed 15% (Bowen et al. 1991). Integrated management of powdery mildew includes the use of resistant varieties, avoiding excessive nutrient applications, and, if needed, the application of a foliar fungicide. Over the last 5 years, producers in Delaware and Maryland have encountered powdery mildew more frequently than in the past, and disease losses have been more common. For example, discussions with local crop consultants indicated that prior to 2011, management of powdery mildew in wheat was not deemed necessary. Since that time, it has developed into an annual issue,

particularly when susceptible varieties are planted near waterways of the Atlantic Ocean. Part of this may be attributed to recent observations that mid-Atlantic populations of Bg can overcome host resistance genes, commonly included in many commercially available wheat varieties in the region (Cowger et al. 2018). Consequently, a reduction in the number of varieties with effective resistance to powdery mildew has resulted in an increase in the use of foliar fungicides for in-season management of this disease.

There are a limited number and classes of fungicide modes of action available for conventional wheat production. The most common fungicide modes of action used for wheat disease management include those belonging to demethylation inhibitor (DMI), succinate dehydrogenase inhibitor (SDHI), and quinone outside inhibitor (QoI) groups (Mueller et al. 2013). These single-site fungicide active ingredients have translaminar and acropetal activity, but mobility differences exist between groups and active ingredients within groups (Latin 2011). Fungicides containing two and three active ingredients are increasingly common as companies strive to prolong the utility of specific active ingredients and minimize issues related to fungicide resistance development in populations of fungal pathogens. In addition, physical differences in fungicide active ingredients and formulation chemistry may affect overall fungicide performance.

There is wide agreement among extension plant pathologists and agronomists that fungicide efficacy is maximized when applied immediately prior to infection by a fungal pathogen (Mueller et al. 2013). However, this situation is rarely encountered in wheat production systems, where fungicides are often applied on a calendar or growth stage system, regardless of existing disease pressure (Sylvester and Kleczewski 2018; Sylvester et al. 2018; Weisz et al. 2011). Identifying fungicides that provide the largest effective control window may help producers with product selections for managing powdery mildew.

The window of efficacy is related to the ability of a fungicide to stop existing infections and to the duration of time that the fungicide provides effective disease suppression. These terms are better defined as the curative and protective activities of the fungicide, and rarely are these characteristics of fungicides studied in a controlled fashion (Ranzi and Forcelini 2013). Studying the characteristics of fungicides on an obligate pathogen such as Bg in the field is challenging, as spores must be generated on living plants under controlled

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conditions and a significant amount of space and appropriate facilities are required to produce inoculum. Furthermore, conidia of Bg are inhibited by the presence of free water, making uniform and efficacious inoculation in the field challenging because spray application may reduce inoculation efficacy. For this reason, the majority of fungicide efficacy studies of powdery mildew in the field rely on natural infection; therefore, accurate assessments of the overall fungicide activity window including curative and protective activities are nearly impossible to ascertain (Bowen et al. 1991; Hardwick et al. 1994; Hunger et al. 2014, 2007; Lipps and Madden 1989).

To determine whether differences in fungicide efficacy for controlling powdery mildew can be detected, a set of controlled studies were conducted in growth chambers at the University of Delaware. Studies evaluated five commercially available fungicides used in wheat production systems in the mid-Atlantic for their curative and protective control of powdery mildew on a soft red winter wheat variety adapted to the mid-Atlantic region. Fungicide-free controls were used for comparison.

Materials and Methods

Experiments were conducted at the University of Delaware Fischer Greenhouse and Growth Chamber Facility located in Newark, Delaware (39.6663° N, 75.74968° W). There were two sets of studies, a curative study (inoculation with powdery mildew prior to fungicide application) and a protective study (fungicide application prior to inoculation with powdery mildew). Curative and protective experiments were conducted separately and therefore are considered independent studies. Curative studies and production of Bg conidia were conducted using the highly Bg-susceptible wheat variety Blueboy (Murphy 1967). For the protective studies, the highly Bg-susceptible wheat variety Roane (Griffey et al. 2001) was used because of insufficient quantities of Blueboy seed at the time of the experiment. Seeds were sown at a rate of 25 seeds/pot into 9-cm² pots filled three-quarters full with pasteurized Jiffy Mix (Jiffy Products

Inc., Lorain, OH) and amended with 3 ml of 12:12:12 (N:P:K) slow release Osmocote fertilizer (ICL Everris NA, Columbus, OH). Pots were placed inside a Percival Scientific Inc. (Perry, IA) model 1-60LLVL growth chamber set to provide 400 photosynthetically active radiation light through cool white fluorescent bulbs set on a 12/12-h day/night cycle. Temperature and relative humidity were maintained at 18°C and 80%, respectively. To minimize cross-contamination, two chambers were used: one was dedicated to the production of Bg inoculum and the maintenance of inoculated plants (Bg chamber), and the other was used for the production of disease-free plants and the maintenance of plants prior to inoculation with Bg (clean chamber). Plants were grown for 10 days after emergence (approximately two leaves exposed) before being used in their respective experiments.

Fungicide and inoculation timing treatments were arranged in a two-factor complete factorial in a generalized randomized block design (GRBD), where experimental run served as the random blocking factor. The two treatment factors were Bg inoculation timing (5 and 3 days before fungicide application [DBA] for curative; and 0, 21, and 42 days after application [DAA] for protective) and fungicide product. Each treatment combination was replicated five times per experimental run, and each of the two studies consisted of two experimental runs. This resulted in a total of 120 (2 runs × 2 timings × 6 treatments × 5 replications) and 180 (2 runs × 3 timings × 6 treatments × 5 replications) experimental units for the curative and protective experiments, respectively.

Powdery mildew treatments. An isolate of Bg collected from Kent County, Delaware, was provided by DuPont Crop Protection (Wilmington, DE). Pilot studies indicated that it was highly virulent on susceptible wheat varieties (N. Kleczewski, *unpublished data*). The isolate was maintained by sequential transfers through flats containing 10-day-old wheat seedlings, maintained in a growth chamber (model AR-75L; Percival Scientific Inc.) under the conditions previously described. Deposition chambers, 61 cm in diameter and 91 cm deep (large polypropylene trash cans), were used to conduct inoculations. Inoculations were performed by exposing plants to conidia from source plants inoculated 14 days prior with Bg. To inoculate plants, 6 to 10 uninfected seedlings were placed within each chamber in such a way that no leaves were pressed against the sides of the chamber and other pots. Then three source plants of wheat containing actively sporulating Bg conidia were inverted above each chamber and gently tapped, releasing conidia into the chamber. The spore inoculation rate was approximately 2,225 spores/cm² as based on the results of pilot studies in which this method was used to deposit spores into deposition chambers containing glass slides placed at the canopy level. Plants were kept in the chamber for 2 min to ensure even and complete spore deposition before removal. After inoculation, pots were transferred to the Bg chamber for incubation. Individual pots were visually rated 10 days postinoculation for percent powdery mildew severity only on the portion of the canopy treated with fungicides. This rating was chosen based on previous studies that indicated that pustules of Bg developed by 10 days after inoculation.

Table 1. Curative and protective experiment timelines for fungicide application, inoculation with *Blumeria graminis*, and disease rating

Day	Experiment ^a	
	Curative	Protective
0	Inoculate all	Spray all Inoculate 0 DAA
3	Spray 3 DBA	
5	Spray 5 DBA	
10	Rate all DBA	Rate 0 DAA
21		Inoculate 21 DAA
31		Rate 21 DAA
42		Inoculate 42 DAA
52		Rate 42 DAA

^a All studies were conducted two times, with five experimental units per fungicide treatment × timing combination per experimental run. DAA = days after fungicide application; DBA = days before fungicide application.

Table 2. Fungicide products and rates assessed for curative and protective activity against *Blumeria graminis* in controlled growth chamber studies

Trade name	Manufacturer	Active ingredient(s)	Mode(s) of action (FRAC code) ^a	%	Rate (liters/ha)
Caramba	BASF	Metconazole	DMI (3)	8.6	0.987
Priaxor	BASF	Fluxapyroxad	SDHI (7)	14.3	0.292
Prosaro	Bayer	Pyraclostrobin	QoI (11)	28.6	0.475
		Prothioconazole	DMI (3)	19.0	
Stratego YLD	Bayer	Tebuconazole	DMI (3)	19.0	0.292
		Prothioconazole	DMI (3)	10.8	
		Trifloxystrobin	QoI (11)	32.3	
Trivapro	Syngenta	Benzovindiflupyr	SDHI (7)	2.9	1.00
		Azoxystrobin	QoI (11)	10.5	
		Propiconazole	DMI (3)	11.9	

^a FRAC = Fungicide Resistance Action Committee, DMI = demethylation inhibitor, SDHI = succinate dehydrogenase inhibitor, and QoI = quinone outside inhibitor.

To ensure that only tissues exposed to the fungicides were rated, a set of plants were sprayed with water-soluble dye the same day as each fungicide treatment. These plants served as visual guides to ensure that only treated portions of the canopy, and not new, untreated growth, were rated. All plants were also rated for chemical injury (burn, chlorosis, stunting) 7 days after fungicide applications, but none was observed. The curative and protective study timelines are presented in Table 1 for clarity.

Fungicide treatments. Fungicides were applied at the rates listed in Table 2. Fungicides and rates were chosen based on relative use in wheat production in the Chesapeake Bay region as indicated through discussions with producers, retailers, and local extension agents. Applications were made using a DeVries (Hollandale, MN) spray chamber set to deliver fungicides at 206.8 KPa, 345.72 cm above the canopy via a single Teejet 80v02 flat fan spray nozzle, resulting in

a 187-liter/ha spray volume. This application method delivered fungicides at rates similar to standard label recommendations. Nontreated controls were treated with water only. Plants were kept at room temperature for 2 h after spray applications, after which they were transferred either to the Bg chamber for incubation or to the clean chamber until inoculation.

Statistical analysis. The curative and protective experiments were analyzed separately but using the same model as described below. Preliminary analysis of this GRBD indicated that the errors were non-normal and more closely followed a Poisson distribution. Therefore, a generalized linear mixed model with a log-link function and Poisson distribution was used to analyze both the curative and protective experiments individually. All analyses were conducted in PROC GLIMMIX (SAS 9.4), and the approach employed is similar to that found in Garrett et al. (2004), Madden et al. (2002), and Shah and Madden (2004). Specifically, the full model used was as follows:

$$\ln(E[Y_{ijk}]) = \eta_{ijk} = \mu + R_i + T_j + F_k + RT_{ij} + RF_{ik} + TF_{jk} + RTF_{ijk}$$

where $\eta_{ijk} = \ln(E[Y_{ijk}])$ is the natural log of the expected value of the observed disease rating corresponding to the i th experimental run (i.e., the random blocking term), the j th inoculation timing, and the k th fungicide treatment; μ is the grand population mean; R_i is the random effect of the i th experimental run, $NID(0, \sigma_R^2)$; T_j is the fixed effect of the j th inoculation timing; F_k is the fixed effect of the k th fungicide treatment; RT_{ij} is the random interaction between experimental run and inoculation timing, $NID(0, \sigma_{RT}^2)$; RF_{ik} is the random interaction between experimental run and fungicide treatment, $NID(0, \sigma_{RF}^2)$; TF_{jk} is the fixed interaction between inoculation timing and fungicide treatment; and RTF_{ijk} is the random three-way interaction between experimental run, inoculation timing, and fungicide treatment, $NID(0, \sigma_{RTF}^2)$. Preliminary analyses also indicated that none of the interactions involving the random experimental run term

Table 3. Results of analysis of curative fungicide studies assessing fungicide performance of five commercially formulated fungicides for controlling powdery mildew on wheat using a generalized linear model analyses with log-link function

Effect ^a	df ^b	F value	P value
Experimental run	1	NA ^c	0.251
Fungicide	5	120.4	<0.001
Inoculation	1	4.0	0.047
Fungicide × inoculation	5	3.6	0.005
Residual	107		

^a Random effects of block and experimental run were not significant, and effects were removed from the analysis.

^b df = degrees of freedom.

^c NA = the F value for the experimental run term is not included because random terms are not included in the type III analysis for fixed effects. By contrast, the P value for the experimental run term was calculated using a Wald approach.

Fig. 1. Mean powdery mildew severities for five fungicides and the nontreated control applied after inoculation of Blueboy wheat. Ratings were made 10 days after inoculation. Different letters indicate significant differences using Tukey's honest significant difference ($\alpha = 0.05$). DBA = days before fungicide application.

were significant ($P > 0.1$ in all cases). Therefore, this model reduced to the following:

$$\ln(E[Y_{ijk}]) = \eta_{ijk} = \mu + R_i + T_j + F_k + TF_{jk}$$

All P values and other findings presented in the results are based on this reduced model. In the case of a significant interaction between inoculation timing and fungicide treatment, an analysis of simple effects with a multiple comparisons type I error adjustment was employed. Treatment combination means (the least-squares means [LSMEANS] of a particular timing-by-fungicide treatment combination) were separated using Tukey's honest significant difference at $\alpha = 0.05$. The percent disease control for the k th fungicide treatment was calculated as follows:

$$\frac{D_{jC} - D_{jk}}{D_{jC}} \times 100\%$$

where D_{jC} is the LSMEAN of the control at the j th inoculation timing and D_{jk} is the LSMEAN of the k th fungicide treatment at the j th inoculation timing. Percent disease control calculations were based on the inverse link scale LSMEANS.

Table 4. Results of analysis of protective fungicide studies assessing fungicide performance of five commercially formulated fungicides for controlling powdery mildew on wheat using a generalized linear model analyses with log-link function

Effect ^a	df ^b	F value	P value
Experimental run	1	NA ^c	0.245
Fungicide	5	152.1	<0.0001
Inoculation	2	257.4	<0.0001
Fungicide $\times$ inoculation	10	11.8	<0.0001
Residual	161		

^a Random effects of block and experimental run were not significant, and effects were removed from the analysis.

^b df = degrees of freedom.

^c NA = the F value for the experimental run term is not included because random terms are not included in the type III analysis for fixed effects. By contrast, the P value for the experimental run term was calculated using a Wald approach.

Results

Curative studies. A significant interaction was detected between fungicide treatment and inoculation timing for powdery mildew severity in the curative studies ($P = 0.005$; Table 3). However, this interaction was attributable to slight and nonsignificant changes in rank among Stratego YLD, Caramba, Prosaro, and Trivapro across the two inoculation timings. Within a particular fungicide treatment, the effect of inoculation timing was found to be nonsignificant in all cases. The percent disease control ranged from 32 to 97% (Fig. 1). Priaxor provided 32 to 58% disease control, whereas all other tested fungicides provided >92% disease control (Fig. 1).

Protective studies. A significant interaction was detected between fungicide treatment and inoculation timing for powdery mildew severity in the protective studies ($P < 0.0001$; Table 4). Overall, the amount of disease control decreased from 0 to 42 DAA; however, the amount of disease control differed by timing and product (Fig. 2). At 0 DAA, percent control was significantly greater in Prosaro (90%), Stratego YLD (89%), and Trivapro (79%) compared with Priaxor (64%) and Caramba (51%).

At 21 DAA, percent control was similar among Prosaro (79%), Stratego YLD (77%), and Trivapro (67%). Prosaro and Stratego YLD provided significantly more control than Caramba (58%), whereas Prosaro provided more control than Priaxor (65%). Caramba and Priaxor provided similar levels of control (Fig. 2).

At 42 DAA, Stratego YLD (68%) provided significantly more control than Caramba (39%), Prosaro (49%), and Trivapro (43%) but statistically similar control to Priaxor (56%). Priaxor provided control similar to Prosaro and Trivapro but greater than Caramba (Fig. 2).

Discussion

In this work, we demonstrated that although commercially available fungicides commonly used in mid-Atlantic wheat production systems provide control of powdery mildew, there are differences in their curative and protective efficacies that may potentially result in variable overall performance. Our data also indicate that the protective effects of foliar fungicides can still be evident up to 42 days after inoculation with Bg under growth chamber conditions.

It is generally recognized that protective treatments provide greater control of fungal plant pathogens, as fungicide active ingredients are present on and within plant tissues prior to the arrival of fungal propagules and prevent any damage resulting from infection and

Fig. 2. Mean powdery mildew severities for five fungicides and the nontreated control applied to Roane wheat prior to inoculation. Ratings were made 10 days after inoculation. Different letters indicate significant differences using Tukey's honest significant difference ($\alpha = 0.05$). DAA = days after fungicide application.

establishment. Our data indicate that all fungicide products tested can provide curative activity of powdery mildew within 5 days of inoculation; however, the amount of control varied with fungicide. Priaxor, the only product tested that does not include a triazole (DMI) active ingredient, provided nearly half as much curative activity as other tested fungicides. These results support those of Ranzi and Forcelini (2013) who assessed the curative effects of DMI, QoI, and DMI+QoI mixtures for controlling *Pyrenophora tritici-repentis* in wheat. In their research, the application of a DMI or DMI+QoI mixture significantly reduced lesion expansion when applied within 5 days of inoculation, with no effects detected for products containing only QoI active ingredients, thus indicating that DMI active ingredients have better curative activity than QoI fungicides. Given the Priaxor results, we hypothesize that fluxapyroxad, the SDHI active ingredient, does not have the curative activity of the DMIs tested. Although some SDHI (Chen et al. 2014; Duan et al. 2019; He et al. 2017; Veloukas and Karaoglanidis 2012) and QoI (Anesiadis et al. 2003) fungicide active ingredients have limited curative activity in some pathosystems, their efficacy is best realized when applied prophylactically (Mueller et al. 2013). It is important to note that in this study, curative activity of the fungicides was only assessed 5 and 3 days after inoculation. This is a relatively short time for the pathogen to colonize and infect plant tissues. In agricultural systems, curative activity is often associated with making a fungicide application after visualizing signs or symptoms of the pathogen on the plant. In the case of powdery mildew in this study, symptoms of disease started to develop by about 8 days after inoculation, and signs were apparent by 10 days after inoculation. Thus, application of the products 10 days after inoculation would have provided an assessment of curative activity more in line with that typically used in field settings and presents an opportunity for continuing research.

In recent years, the residual or protectant activity of fungicides has been a talking point for many in the agricultural industry. Excellent fungicide residual allows producers to minimize the number of applications made during the growing season. Trivapro, for example, has been advertised as having at least a 42-day residual in field studies (Syngenta Crop Protection 2017). Our data indicate that under the conditions encountered in this study, all tested fungicides significantly reduced the development of powdery mildew at 42 DAA, compared with the control. Of the fungicides tested, Priaxor, Stratego YLD, and Trivapro provided the greatest protective activity. Although the exact reason for the difference in performance in this study is not known, we speculate that it is related to factors associated with the active ingredients (i.e., degradation by the plant, mobility, relative concentration, potency) as well as factors related to inactive ingredients contained within the formulated products. In the field, rainfastness may influence fungicide efficacy (Andersen et al. 2014). Rainfastness can be influenced by several factors, including the amount of rain and when it occurs in relation to fungicide application, product formulation, surfactants, and physical and chemical properties of the fungicide active ingredient (Gaskin and Steele 2009; Hislop 1967; Hunsche et al. 2006; Ivic 2010; Krogh et al. 2003; Vicent et al. 2007). In addition, fungicide efficacy can also be impacted by the quantity and quality of light (Haggi et al. 2002; Kouras-Hadef et al. 2018; Wen et al. 2000), temperature at application, vapor phase activity, plant part being treated, microbial activity, droplet size, and fungicide mobility (Baumeister et al. 2002; Bedos et al. 2010; Leistra and Wolters 2004). In our studies, the impacts of these factors on the degradation of fungicidal components were likely minimized, and overall length of activity was therefore more pronounced than what would be expected in typical field conditions, owing to the lack of overhead watering and use of artificial lighting. Regardless of these limitations, our data indicate that although many of the tested fungicides share common modes of action, there are subtle differences in product efficacy that could affect overall performance and utility for managing powdery mildew in a field setting. Additional field research is needed to better understand the overall longevity and efficacy of these products and how this relates to the duration of control of powdery mildew in wheat.

Powdery mildew is not the only disease encountered in wheat production systems in the mid-Atlantic; other frequently encountered diseases include *Parastagonospora* leaf and glume blotch, tan spot, and *Fusarium* head blight (Sylvester and Kleczewski 2018; Sylvester et al. 2018). The overall effectiveness of fungicides in wheat production systems in the region will ultimately depend on the overall efficacy of the chosen fungicide across all commonly occurring, yield-limiting fungal diseases and on product cost. Thus, we suggest that future studies assess product efficacy under controlled conditions similar to those described here to better understand how commonly used fungicides differ in overall efficacy and duration of control across commonly encountered pathosystems.

This work demonstrates that foliar fungicides used in mid-Atlantic wheat production systems are effective for controlling powdery mildew. The effectiveness of products for managing this disease may vary slightly depending on the product used and the timing of application relative to infection. Overall, application of all fungicide products tested in this study significantly reduced powdery mildew relative to nontreated controls. However, future studies are needed to determine the efficacy and profitability of using these products in the field.

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