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Fungicide and cultivar effects on the development and temporal progress of wheat blast under field conditions

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ABSTRACT

The epidemiology of wheat blast, caused by the *Triticum* pathotype of *Pyricularia oryzae*, is poorly understood, making it very difficult to manage. We reported on the individual and combined effect of host resistance and fungicide application for managing wheat blast disease on spikes. Two field experiments (Exp. 1 and Exp. 2) were conducted in a region of Brazil where blast is not known to be endemic to evaluate its development as influenced by fungicide and host resistance. Plots of wheat cultivars BR-18 (partially resistant) and Guamirim (susceptible) were either treated with the fungicide epoxiconazole + pyraclostrobin or left non-treated, and then inoculated with a spore suspension of *P. oryzae* at mid-anthesis. Spike blast incidence and severity, quantified at regular intervals after inoculation, increased over time, and fungicide and cultivar had statistically significant effects ($P < 0.005$) on both measures of disease and their temporal rates of progress. Relative to Guamirim-non-treated, BR-18-non-treated (resistance alone) led to 44 and 64% control of final incidence and severity, respectively, in Exp. 1, and 3 and 49% control, respectively, in Exp. 2. Guamirim-treated (fungicide alone) led to 65% control of incidence and 77% control of severity in Exp. 1, and 64% control of incidence and 95% control of severity in Exp. 2. For both incidence and severity, fungicide and resistance alone also reduced the temporal rate of progress relative to the susceptible non-treated. However, the greatest overall efficacy was observed when resistance and fungicide were combined, with over 70 and 90% control of final incidence and severity, respectively, and over 75% reduction in the temporal rate of spike blast progress. Based on percent control, the integrated effect of resistance and fungicide was additive for incidence, severity, and their temporal rates of progress, demonstrating the value of combining the two strategies to manage spike blast.

1. Introduction

The development of wheat blast, caused by the fungus *Pyricularia oryzae* (Cooke) Sacc., is favored by rainy weather, temperatures ranging from 21 to 27 °C, cloudy days, and high relative humidity (Goulart et al., 2007). Under such favorable conditions, yield losses as high as 60% have been reported (Goulart et al., 2007). *P. oryzae* infects all above-ground parts of the wheat plants, including leaves, culms, and spikes (Igarashi et al., 1986). On the leaves, typical symptoms are elliptical or roundish lesions with dark-brown margins and grayish centers (Goulart et al., 2007). On spikes, symptoms appear as bleaching and death of infected tissues and dark discoloration of the rachis (Goulart et al., 2007). Although the leaf-blighting stage of wheat blast may affect grain yield and quality, the greatest damage occurs when the spikes are infected, since this often leads to reduced translocation of nutrients to the grains, causing them to become shriveled, small, and lightweight (Goulart et al., 2007).

Since first being reported in 1985 in the state of Parana, Brazil (Igarashi et al., 1986), wheat blast has become widely distributed across all major wheat-producing areas in Brazil and some neighboring countries (Maciel et al., 2014). Wheat blast is now considered one of the biggest obstacles to the expansion of wheat production in Brazil (Maciel et al., 2014). Current management

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strategies for minimizing losses caused by wheat blast include the use of resistant cultivars, when available, and fungicide application (Maciel, 2011; Castroagudín et al., 2015). Although most commercial cultivars are susceptible to blast, BR-18 terena, BRS 229, and MGS3 Brilhante are considered to be moderately resistant (Maciel et al., 2008; Cruz et al., 2010). However, due to the high genetic diversity found in *P. oryzae* populations, partial resistance is generally not durable (Maciel et al., 2014). Of the foliar fungicides recommended for wheat blast management, the Quinone Outside Inhibitors (QoI), marketed either as single active ingredients or as premixes with demethylation inhibitors (DMI), are the most widely used in Brazil (Maciel, 2011), but their efficacy has been highly variable (Goulart et al., 1996; Urashima et al., 2005; Maciel, 2011). This variability could be attributed, at least in part, to the extensive use of fungicides for wheat blast control over the last few years, which likely resulted in a selection for QoI resistance in *P. oryzae* populations (Castroagudín et al., 2015).

Even after more than 30 years of research, there are still many unanswered questions about the epidemiology and management to wheat blast, particularly the spike blast stage of the disease. For instance, the optimum plant growth stage for spikes infection is still a subject of debate; the temporal change in spike blast intensity has not be thoroughly investigated; the epidemiological importance of leaf blast for spike blast development is largely unknown; and further research is needed to better characterize the incubation and latent periods of spike blast. A thorough understanding of these epidemiological components of blast is important for establishing the optimum time and frequency of fungicide application for disease management. Knowledge gaps in this area may be among the reasons why there have been mixed reports, both anecdotal and published, regarding the efficacy of fungicides against wheat blast on the spikes under field conditions. For instance, Rocha et al. (2014) reported that two applications of tebuconazole, epoxiconazole + pyraclostrobin, or tebuconazole + trifloxystrobin, the first at Zadoks 45 (boot) and the second at Zadoks 65 (mid anthesis), reduced leaf blast incidence (as area under the progress curve) and severity, but were ineffective against spike blast severity. Contrastingly, however, Pagani et al. (2014) reported that two applications of the same three fungicides (the first at early heading followed by a second at the milk growth stage) reduced spike blast severity by 35–72%. Furthermore, Rocha et al. (2014) observed that the magnitude of fungicide effects on leaf blast incidence varied among the four wheat genotypes evaluated in the study.

The primary objectives of this study were to: (*i*) evaluate the effects of the fungicide 13.3% epoxiconazole + 5% pyraclostrobin and cultivar resistance on incidence and severity of wheat blast on spikes under field conditions and (*ii*) characterize the temporal progress of incidence and severity of wheat blast on spikes as influenced by partial resistance and fungicide treatment.

2. Materials and methods

2.1. Plot establishment, fungicide treatment and inoculation with *P. oryzae*

Two field experiments were conducted in an experimental area of the Federal University of Viçosa, Viçosa, Brazil, located in the southeastern region of the state of Minas Gerais (20°44′44″S, 42°50′59″W, and 661 m above sea level). The first experiment was carried out from June to September 2013 and the second from August to November 2013. Plots were planted using a Kincaid planter on June 1 and August 8 in experiments 1 and 2, respectively, at a population density of 70 plants per meter of row. Each plot (experimental unit) consisted of five 5-m-long rows, spaced 0.2 m apart, corresponding to a total plot area of 5 m^2. The distance between adjacent plots was 1 m. All plots were managed and maintained according to conventional Brazilian wheat production practices, including fertilizer application before planting based on soil chemical analysis.

The experimental design was a randomized complete block, with a 2 $\times$ 2 factorial arrangement of fungicide treatment and cultivar in four replicate blocks. Separate plots of wheat cultivars BR-18 (moderately resistant) and Guamirim (susceptible) were either treated with the fungicide 13.3% epoxiconazole + 5% pyraclostrobin (Opera, Basf S.A.- São Paulo, Brazil) at growth stage 65 (mid-anthesis, Zadoks et al., 1974) at a rate of 0.5 L ha^{-1} or left non-treated. Applications were made using a CO_2 pressurized backpack sprayer (3.1 $\times$ 10^5 Pa) with Teejet 110.03 nozzles, at a volume of 200 L ha^{-1}.

Approximately 48 h after fungicide application, plots were spray-inoculated with a suspension containing 10^5 conidia/mL of isolate UFV/DFP-*Po*01 of *P. oryzae*. Leaves and spikes were inoculated at 18:00 h with approximately 1000 mL of the inoculum applied to each plot using a CO_2 pressurized backpack sprayer (3.1 $\times$ 10^5 Pa) with Teejet 110.03 nozzles. Two hours before inoculation, all plots were mist-irrigated for 10 min to increase humidity and enhance infection.

Weather data (precipitation, average relative humidity, and maximum, average and minimum temperature) were obtained from an onsite weather station.

2.2. Blast assessment, grain yield and data analysis

Incidence and severity of wheat blast were assessed on 40 arbitrarily selected spikes in each plot at 10, 14, 18, and 22 days after inoculation (dai). Incidence was rated as the mean percentage of spikes diseased out of the 40 spikes sampled ([number of diseased spikes/40] $\times$ 100), whereas severity was quantified as the mean proportion of diseased spikelet per spike ([diseased spikelets/total spikelets rated] $\times$ 100). Spikes in all rows of each plot (an area of approximately 5 m^2) were harvested on 22 September 2013 in experiment 1, whereas only those in the three center rows of each plot, representing an area of approximately 3 m^2, were harvested on 27 November in experiments 2. Spikes were threshed, grains weighed, and plot yield was estimated in g m^{-2} and then converted to kg ha^{-1} at 12% moisture.

2.2.1. Effect of cultivar resistance and fungicide treatment on incidence and severity of wheat blast

All incidence and severity data were arcsine-square-root-transformed prior to analysis to stabilize variance, and each experiment and measure of disease was analyzed separately. To evaluate the integrated effects of cultivar resistance and fungicide treatment on wheat blast, models were fitted to the arcsine-square-root-transformed incidence and severity data with cultivar, fungicide treatment, and disease assessment time as categorical fixed effects and block as a random effect. Since incidence and severity data were collected as temporal repeated measures on the same experimental units and as such were correlated in time (Littell et al., 2006), the random *_residual_* statement and *type* option in GLIMMIX were used to account for, and model, the covariance structure of the within-subject data. Models were fitted using the GLIMMIX procedure of SAS (Littell et al., 2006). The *slice* option in the *lsmeans* statement of GLIMMIX was then used to compare the least squares means among cultivars $\times$ fungicide application combinations at each assessment times (growth stages). The model fitted to the data can be written as: