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Comparison of single- or multi-active ingredient fungicides for controlling Fusarium head blight and deoxynivalenol in Brazilian wheat

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ABSTRACT

In this study, we gathered data on Fusarium head blight (FHB) severity, deoxynivalenol (DON), and wheat yields from 19 cooperative fungicide trials conducted in Southern Brazil over five growing seasons (2017–2021). We tested three premixes of Quinone Outside Inhibitors (QoIs) + demethylation inhibitors (DMIs) (PYRAclostrobin + METConazole, TEBUconazole + TriFLoXystrobin, and TriFLoXystrobin + PROThioconazole), one triple premix of QoI + DMI + succinate dehydrogenase inhibitors (SDHI) (TriFLoXystrobin + PROThioconazole + BIXaFen), and two single active ingredients (METC [DMI] and CARBendazim [benzimidazole; MBC]) applied three times, beginning at the flowering stage and continuing every 7–12 days. We fitted a network meta-analysis model to the log of the means of FHB index and DON content data and to the non-transformed mean yield for each treatment, including the untreated control. Disease (FHB index) reduction estimates ranged from 41.5% (TEBU + TFLX) to 62.8% (METC); the latter did not differ from PYRA + METC (56.1%). Likewise, the mean estimates of percent DON reduction were higher for METC (65.1%) and PYRA + METC (58.3%). These two treatments were followed by TEBU + TFLX (50%), which was not statistically different from CARB (48%) and TFLX + PROT (45.2%), but differed from TFLX + PROT + BIXF (39.3%). Lastly, the yield response was higher for TFLX + PROT + BIXF (643 kg/ha), which differed from all other treatments, including METC (505.9 kg/ha), PYRA + METC (477.8 kg/ha), TFLX + PROT (455.3 kg/ha), CARB (453.2 kg/ha), and TEBU + TFLX (403.4 kg/ha). The results of this meta-analysis are crucial for choosing fungicides when planning programs aimed at reducing both FHB and DON levels in wheat.

1. Introduction

The globally significant wheat disease known as Fusarium head blight (FHB) or wheat scab, significantly impacts wheat cultivation, resulting in not just reduced grain yield but also the production of harmful mycotoxins (McMullen et al., 2012). The infection is primarily caused by species of the *Fusarium graminearum* species complex, predominantly *F. graminearum* (Del Ponte et al., 2015). Research has

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indicated that, in Brazil, the reduction in wheat production attributable to FHB is estimated to be between 3% and 25% over more than a quarter-century (Duffeck et al., 2020). Deoxynivalenol (DON), a mycotoxin from the type-B trichothecene group, is of considerable concern due to its high prevalence in wheat grains. Mycotoxins like DON and nivalenol, along with zearalenone, are regularly found in wheat produced commercially in Brazil, as shown by multiple toxin detection studies (Del Ponte et al., 2012; Duffeck et al., 2017). The level of mycotoxin accumulation can be affected by a variety of factors, such as climate, rotation of crops, tillage techniques, resistance of the cultivar, and fungicide treatments (Ellner 2005; Feksa et al., 2019; Mesterházy et al., 2003; Paul et al., 2008, 2018).

Fungicide treatments play a crucial role in controlling FHB. It is advised to apply fungicides at full bloom and to use less susceptible crop varieties to optimize disease control (Mesterházy et al., 2003; Wegulo et al., 2011, 2015; Willyerd et al., 2012). Among several fungicides, demethylation inhibitors (DMIs) (classified under the Fungicide Resistance Action Committee [FRAC] group 3), are routinely top-rated for their efficacy in reducing FHB and DON (Machado et al., 2017; Mesterházy et al., 2011, 2018; Paul et al., 2008). In the context of the United States, for instance, the use of metconazole or prothioconazole, either solely or in combination, has led to a significantly larger reduction in FHB and DON levels compared to tebuconazole and propiconazole when used separately (Paul et al., 2008, 2018). A meta-analysis conducted in Brazil showed that the application of tebuconazole led to markedly higher wheat yields (+100 kg/ha on average) than when propiconazole and carbendazim were used (Machado et al., 2017). Benzimidazole (MBCs) fungicides, grouped under FRAC group 1, especially carbendazim, are considered a cost-efficient alternative, albeit they appear to show better results when FHB severity is relatively low (Machado et al., 2017; Mesterházy et al., 2011). Carbendazim has been in used in Brazil since the early 1980s for managing FHB (Deuner et al., 2011). Recently, the potential of a novel succinate dehydrogenase inhibitor (SDHI) (FRAC group 7) fungicide, pydiflumetofen, either standalone or in combination with DMIs, has been underscored for its promising activity against FHB (Edwards 2022; Singh et al., 2021; Xia et al., 2021).

Fungicides classified as Quinone Outside Inhibitors (QoIs), falling under the Fungicide Resistance Action Committee (FRAC) group 11, are typically not advised for the management of Fusarium head blight (FHB) and reduction of deoxynivalenol (DON) because they exhibit less effectiveness compared to triazoles (Bolanos-Carriel et al., 2020; Feksa et al., 2019; Magan et al., 2002; Pirgozliev et al., 2002). However, recent investigations in Brazil have shown mixed outcomes when comparing QoI-enhanced combinations with single active constituents or different combinations. For instance, a study consisting of four trials in the state of Rio Grande do Sul indicated noticeable differences in yield for the combination of pyraclostrobin and metconazole versus using metconazole by itself in some instances (Spolti et al., 2013). In contrast, a four-year study conducted in Paraná State showed that DMIs and MBCs outperformed DMI + QoI combinations concerning FHB and DON management (Feksa et al., 2019). Additionally, there have been reports of either slight reductions or even elevations in DON levels in wheat grains in comparison to untreated samples, especially in fields that were treated primarily with the QoI fungicide azoxystrobin (Ellner 2005; Feksa et al., 2019; Mesterházy et al., 2003; Simpson et al., 2001).

The exact processes behind the increase in mycotoxin production induced by QoI fungicides remain somewhat obscure. Nevertheless, several QoIs such as coumoxystrobin, picoxystrobin, fluoxastrobin, azoxystrobin, fenaminstrobin, and pyraclostrobin have been linked to the production of DON by promoting the expression of Tri5 and Tri6 genes and boosting the production of acetyl-CoA (Duan et al., 2020). Despite the common advice against employing QoIs to control FHB, combinations of QoIs and DMIs have demonstrated certain advantages. These include providing prolonged defense against foliar diseases like powdery mildew, tan spot, and various rusts (Barro et al., 2017; Blandino et al., 2006; Paul et al., 2018; Ransom and McMullen 2008; Willyerd et al., 2012). Therefore, maintaining the longevity of the flag leaf through the application of QoI + DMI mixes may contribute to higher yields, particularly in environments that are conducive to FHB (Blandino et al., 2011; Wegulo et al., 2011).

The performance of fungicides can vary widely depending on environmental conditions and is influenced by numerous factors such as the intensity of epidemics, resistance of the cultivar, mode of action of the fungicide, timing and number of applications, and the technology used to apply it (Mesterházy et al., 2011, 2018). In Brazil, the effect of fungicides have been examined for several years, mainly on FHB reduction. Still, the development of a cooperative fungicide trials (CFTs) network, in collaboration with the industry since 2011, has generated crucial insights into creating effective fungicide strategies against FHB (Barro et al., 2021; Machado et al., 2017). The primary goal of the CFTs is not to base a regional recommendation of fungicide program because sequential sprays of the same fungicide is not encouraged due to fungicide resistance issues (Hollomon 2015). Although annual summaries are distributed through technical reports (Santana et al., 2012, 2014, 2016a, 2016b, 2016c, 2019a, 2019b, 2020), the analysis of the data, either at the trial level or when combining all trials within a year, does not provide a clear answer on the most effective methods to control FHB, specifically for reducing DON. To address these discrepancies, meta-analysis is a valuable strategy that enables the estimation of the significance, magnitude, and uncertainty of the effects of a given treatment (Madden and Paul 2011; Madden et al., 2016). This methodology has been increasingly adopted to summarize the impact of fungicides in managing plant diseases, including in FHB research (Barro et al., 2021; Machado et al., 2017; Paul et al., 2008, 2018).

In this study, we collected FHB index, DON content, and wheat grain yield data from the CFTs. The dataset spanned five years (2017–2021) of experiments conducted at seven locations in two wheat-producing states in southern Brazil (Rio Grande do Sul and Paraná). Our primary objective was to obtain meta-analytic estimates of wheat FHB control efficacy, DON reduction, and wheat yield response to a set of commercial fungicides labeled for FHB control in Brazil.

2. Materials and methods

2.1. Data source and criteria for trial and fungicide selection

Data were obtained from 19 field trials conducted by researchers of the FHB cooperative fungicide trials (CFTs) during five years (seasons) (2017–2021) across seven municipalities in the Brazilian states of Rio Grande do Sul (RS) and Paraná (PR). FHB epidemics occurred naturally (without inoculation of the pathogen). Moderately susceptible and moderately resistant wheat cultivars adapted to the region were used in the experiments (data not shown) and all agronomic practices (fertilization, weed and pest control) were performed according to regional recommendations. The trials were conducted following a completely randomized block design with four replications where a plot of 12 m^2 was a replication. All fungicides were applied three times, starting at the heading stage (60 of Zadoks growth stages) (Zadoks et al., 1974), and following 7–12 days apart. The use of three sprays was done with the sole purpose of comparing the efficacy of the fungicides under optimized conditions for control. A backpack sprayer pressurized by CO_2 calibrated to spray 200 L ha^{-1} was used to perform the fungicide applications.

FHB incidence (INC) (proportion of diseased head) and conditional severity (SEV) (proportion of diseased spikelets in a diseased head) (Stack and McMullen 1998) were visually assessed in 1 m-lines at each one of the three central rows of the plot during wheat grain soft dough stage (85 of Zadoks growth stages) (Zadoks et al., 1974). FHB index (IND) was calculated as IND = (INC * SEV)/100. At least 4 m^2-plants were harvested at full maturity. Grain weight and moisture were obtained for each treatment plot (fungicide + untreated). Crop yield was expressed in kg/ha at 13% moisture. The DON content was determined by taking a sample of 300 g of wheat grains per plot and using the AgraQuant® Deoxynivalenol 0.20/5.0 ELISA kit (Duffeck et al., 2017).

To be included in the analysis, a fungicide treatment should have been tested in at least nine trials conducted in at least four years and compared with an untreated check in the same trial. Six fungicides met the criteria, including three QoI + DMI premixes, one triple premix of QoI + DMI + SDHI and two single active ingredients (DMI and MBC) (Table 1). After treatment selection, the number of trials differed among the three response variables (FHB index, DON content and yield). There were 13 trials for FHB index and DON content, and 19 trials for yield because FHB index and DON were not measured in all trials.

2.2. *Meta-analytic model*

We obtained estimates of control efficacy on the disease severity index, DON, and yield response by fitting the data to an arm-based network model. This method is also referred to as a two-way unconditional linear mixed model, and involves fitting the model directly to treatment means (either absolute or log-transformed) (Machado et al., 2017; Madden et al., 2016; Paul et al., 2008). The means of the FHB index and DON underwent log transformation, while no transformation or standardization was necessary to obtain the mean absolute difference in yield due to the data's statistical properties (Fig. S1). Equation (1) represents the arm-based model:

$$Y_i \sim N(\mu, \Sigma + S_i) \tag{1}$$

where Y_i is the vector of L (log of the means of FHB index or DON) or absolute yield for the six treatments plus the untreated check for the ith study, μ is a vector representing the mean of Y_i across all studies, Σ is a 7 × 7 between-study variance-covariance matrix (for the seven treatments, including the untreated check), and S_i is a within-study variance-covariance matrix for the ith study. N indicates a multivariate normal distribution.

The within-study variability (sampling variance) of L and D was calculated from the mean square error (MSE) obtained from a linear model fitted to raw data at each individual trial, as described in previous studies (Machado et al., 2017; Paul et al., 2008, 2010). The within-study variability is required to weight studies based on the inverse function of the sampling variance (Paul et al., 2008). An unstructured (*UN*) matrix Σ was used and maximum likelihood estimation models were fitted to the data using the *rma. mv* function of *metafor* package (Viechtbauer 2010) of R (R Core Team 2020).

Estimates of percent FHB control ($\overline{C}$) and percent DON reduction ($\overline{R}$) were calculated by taking the differences of mean log of the response ratio ($\overline{L}_{IND}$ and $\overline{L}_{DON}$) which equals the ratio of the two means (Paul et al., 2008). The $\overline{C}$ and $\overline{R}$ values and their 95% confidence intervals (Cis) were obtained by back-transforming $\overline{L}_{\mathrm{IND}}$ and $\overline{L}_{\mathrm{DON}}$ and the respective upper and lower limits of their 95% Cis as described in Equations (2) and (3).

$$\overline{C} = (1 - (exp(\overline{L}_{IND})\ )\ x\ 100\ ) \tag{2}$$

$$\overline{R} = (1 - (exp(\overline{L}_{DON})\ )\ x\ 100\ ) \tag{3}$$

Table 1
Fungicide treatments applied for controlling Fusarium head blight in wheat, evaluated in 19 fungicide trials conducted from 2017 to 2021 across two Brazilian states (PR and RS).

Fungicide a.i.	Chemical group[a]	Study code	Commercial name	Dose[b]	Grams (a.i.)/ha
untreated	–	CHECK	–	–	–
carbendazin	MBC	CARB	Bendazol	0.80	250
metconazole	DMI	METC	Caramba	1.00	90
pyraclostrobin + metconazole	QoI + DMI	PYRA + METC	Opera Ultra	0.75	97.5 + 60
tebuconazole + trifloxystrobin	DMI + QoI	TEBU + TFLX	Nativo	0.75	75 + 150
trifloxystrobin + prothioconazole	QoI + DMI	TFLX + PROT	FOX	0.50	75 + 87.5
trifloxystrobin + prothioconazole + bixafen	QoI + DMI + SDHI	TFLX + PROT + BIXF	Fox XPRO	0.50	75 + 87.5 + 62.5

[a] MBC = methyl benzimidazole carbamate; QoI = Quinone-outside inhibitors; DMI = Sterol demethylation inhibitor; SDHI = succinate dehydrogenase inhibitors.

[b] Dose (L/ha) for each fungicide.

The yield difference ($\overline{D}$) was calculated directly after model fitting by subtracting estimated means of fungicide treatment and untreated check (Madden et al., 2016).

In multi-arm network meta-analyses, it is crucial to evaluate inconsistency, which is the degree to which various evidence sources are compatible (Higgins et al., 2012). The most significant source is known as "design inconsistency," and a design-by-treatment interaction offers a valuable general framework for examining inconsistency (Higgins et al., 2012; Piepho 2014; Madden et al., 2016). To determine the significance of the treatment × design interaction, we employed a factorial-style ANOVA model, which was assessed using the Wald test statistic. The null hypothesis posits that the network is consistent (Piepho 2014; Madden et al., 2016). In the trials reporting the FHB index, six distinct designs (where design refers to the treatment set in the trial) were discovered, while five designs were identified for DON reporting and four designs for wheat grain yield response reporting (Table S1).

3. Results

3.1. *FHB index, DON content and yield data at the trial level*

FHB index in the untreated check plots ranged from 2 to 45.7% (median 11.3%) (Fig. 1). Over the years, the lowest (2.6%) and the highest (16.4%) median FHB index in the untreated check were recorded in the 2020 and 2021 seasons, respectively (Fig. 1A). Additionally, the highest median of the FHB index was observed in the state of RS (11.4%), almost three times higher than in the state of PR (3.4%) (Fig. 1B). On the other hand, DON content ranged from 236.9 to 5016.8 μg/kg (median 2622.7 μg/kg). The highest median in DON content (3798 μg/kg) was observed during the 2017 growing season (Fig. 1D). DON content was very similar between states with a median of 2623 μg/kg for RS, and 2421 μg/kg for PR state (Fig. 1E). Finally, the baseline yield ranged from 1354 to 5194 kg/ha (median 3424 kg/ha) across the trials. Baseline yields were generally higher during the 2021 growing season (median 4825 kg/ha) and lower during the 2018 season (median 1986 kg/ha) (Fig. 1G). Across states, RS reported the highest median (3853 kg/ha) compared to PR state (2205 kg/ha) (Fig. 1H). There was a general trend of decreased FHB index and DON content as well as increased yield in the fungicide treatments compared with the untreated check (Fig. 1C,F, I).

3.2. *Percent FHB control*

Overall estimates of percent control efficacy ($\overline{C}$), obtained from back-transforming differences of the estimates of log of FHB index ($\overline{LIND}$) between the fungicide-treated and untreated plots ranged from 41.5 to 62.8% across the treatments. Only METC resulted in percent control above 60% on average and it was not significantly different from PYRA + METC (56.1%) ($P = 0.0933$). The latter was not statistically different ($P > 0.05$) from TFLX + PROT (53.4%) and TFLX + PROT + BIXF (48%). This latter group was followed by CARB (42.7%) and TEBU + TFLX (41.5%), which did not differ statistically between them (Table 2). The difference in percent control efficacy between the most and least effective fungicide was 21 percentage points. The Wald test determined that network consistency was significantly affected by the study design ($P = 0.0165$).

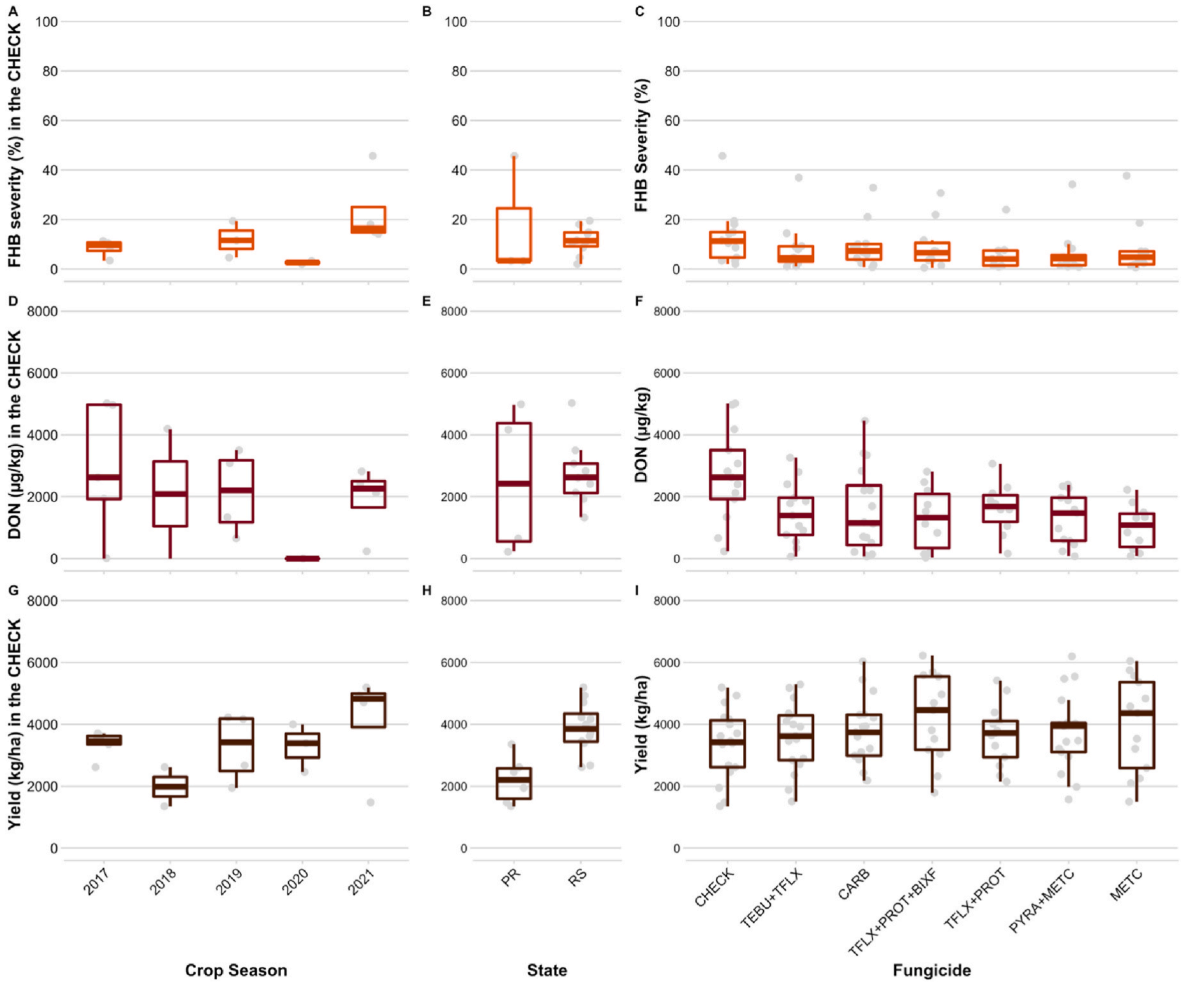

Fig. 1. Box plots for the within-season variation across trials and by state in the untreated check, and means for a set of fungicide treatments of FHB severity (%) (**A–C**), DON (μg/kg) (**D–F**) and wheat grain yield (kg/ha) (**G–I**) obtained from 19 trials conducted during 5 years across two Brazilian states (PR and RS). The thick horizontal line inside the box represents the median, the limits of the box represent the lower and upper quartiles, and the circles represent yearly means of each treatment (See Table 1).

Table 2

Overall means and respective confidence intervals of log response ratio and calculated percent control of Fusarium head blight (FHB) relative to untreated check provided by six fungicides evaluated in 13 independent trials conducted across two Brazilian states (PR and RS) during 5 growing seasons (2017–2021).

Fungicide[a]	k[b]	Effect Size					FHB control (%)		
		$\overline{L}_{\mathrm{SEV}}$	SE ($\overline{L}_{\mathrm{IND}}$)	CI_L[c]	CI_U[c]	*P value*	$\overline{C}$	CI_L[c]	CI_U[c]
METC	10	−0.9901	0.1603	−1.3043	−0.6760	<0.0001	62.8	49.1	72.8
PYRA + METC	12	−0.8246	0.1528	−1.1240	−0.5251	<0.0001	56.1	40.8	67.5
TFLX + PROT	9	−0.7640	0.1481	−1.0544	−0.4737	<0.0001	53.4	37.7	65.1
TFLX + PROT + BIXF	10	−0.6542	0.1252	−0.8996	−0.4089	<0.0001	48.0	33.5	59.3
CARB	12	−0.5573	0.1051	−0.7633	−0.351	<0.0001	42.7	29.6	53.3
TEBU + TFLX	12	−0.5376	0.0886	−0.7113	−0.3640	<0.0001	41.5	30.5	50.8

[a] See Table 1 for complete information of the evaluated fungicides.

[b] Number of trials that each fungicide was evaluated.

[c] Upper (CI_U) and lower (CI_L) limits of the 95% confidence interval around $\overline{L}_{\mathrm{IND}}$ and $\overline{C}$.

3.3. *Percent DON reduction*

The mean estimates of percent DON reduction ($\overline{R}$), obtained from back-transforming differences of the estimates of log of DON content ($\overline{L}_{DON}$) between the fungicide-treated and untreated plots, ranged from 39.3 to 65.1%. METC was the most effective in reducing DON content (>60%), and did not differ from PYRA + METC (58.3%). Those two treatments were followed by TEBU + TFLX (50%), which was not statistically different ($P > 0.05$) from CARB (48%) and TFLX + PROT (45.2%), but differed ($P = 0.0302$) from TFLX + PROT + BIXF (39.3%) (Table 3). The difference in percent control reduction of DON content between the most and least effective fungicide was 26 percentage points. The Wald test determined that network consistency was significantly affected by the study design ($P < 0.0001$).

Table 3
Overall means and respective confidence intervals of log response ratio ($\bar{L}_{DON}$) and calculated percent reduction of DON relative to untreated check provided by six fungicides evaluated in 13 independent trials conducted across two Brazilian states (PR and RS) during 5 growing seasons (2017–2021).

Fungicide[a]	*k*[b]	Effect Size					DON reduction (%)		
		$\bar{L}_{DON}$	SE ($\bar{L}_{DON}$)	CI_L[c]	CI_U[c]	*P value*	$\bar{R}$	CI_L[c]	CI_U[c]
METC	9	−1.0540	0.1232	−1.2954	−0.8126	<0.0001	65.1	55.6	72.6
PYRA + METC	13	−0.8770	0.0977	−1.0684	−0.6855	<0.0001	58.3	49.6	65.6
TEBU + TFLX	13	−0.6934	0.0945	−0.8786	−0.5081	<0.0001	50.0	39.8	58.4
CARB	13	−0.6546	0.1523	−0.9530	−0.3562	<0.0001	48.0	29.9	61.4
TFLX + PROT	10	−0.6021	0.0481	−0.6963	−0.5078	<0.0001	45.2	39.8	50.1
TFLX + PROT + BIXF	9	−0.5008	0.0767	−0.6511	−0.3504	<0.0001	39.3	29.5	47.8

[a] See Table 1 for complete information of the evaluated fungicides.
[b] Number of trials that each fungicide was evaluated.
[c] Upper (CI_U) and lower (CI_L) limits of the 95% confidence interval around $\bar{L}_{DON}$ and $\bar{R}$.

3.4. *Yield response*

The mean estimates of yield difference ($\bar{D}$) between fungicide-treated and the untreated plots ranged from 403 to 643 kg/ha among the fungicide treatments. Yield response values as high as above 600 kg/ha were estimated only for TFLX + PROT + BIXF (643 kg/ha) which differed from all the other treatments ($P < 0.05$). The latter was followed by METC (505.9 kg/ha), which did not differ from PYRA + METC (477.8 kg/ha), TFLX + PROT (455.3 kg/ha), CARB (453.2 kg/ha) and TEBU + TFLX (403.4 kg/ha) ($P > 06$) (Table 4). The difference between the highest and lowest estimated yield means was 240 kg/ha. The Wald test for the treatment × design interaction showed that the network was inconsistent ($P < 0.0001$).

In general, the pattern of the relationship between disease control efficacy and DON reduction was consistent. As shown previously, the most effective treatments in reducing not only disease severity but also DON content were METC and the premix PYRA + METC, which performed the higher yields together with TFLX + PROT + BIXF (Fig. 2).

4. Discussion

This study provides updated information on managing FHB and reducing DON levels with fungicides in Brazil over the last five growing seasons (2017–2021) in the two main wheat-producing states, Paraná (PR) and Rio Grande do Sul (RS). On average, we found that the most effective fungicides for reducing both disease severity and DON content were METC and the premix PYRA + METC. The triple premix TFLX + PROT + BIXF exhibited poor performance in decreasing DON but resulted in increased wheat yields compared to untreated conditions.

Our findings revealed that metconazole applied individually led to the highest level of disease control (62.8%). Greater efficacy (>70%) was reported for METC during a four-year study in southern Paraná (Feksa et al., 2019), where the fungicide was applied after pathogen inoculation, unlike our dataset from natural epidemics. These results also support the superior performance of METC in controlling FHB in the United States (Paul et al., 2018). The control efficacy estimates for CARB (42.7%), the other single active ingredient utilized in this study, were lower than those from a previous meta-analysis (55% on average) that assessed data from 2000 to 2015 (Machado et al., 2017). Nonetheless, our findings align with earlier studies where CARB was reported to be less effective than metconazole (Chen et al., 2012; Mesterházy et al., 2003).

Our control efficacy estimates for the premix PYRA + METC (56.1%) were lower than those reported in a two-year study (62%) conducted in northern RS state, Brazil (Bonfada et al., 2019), and a four-year study (64.5%) conducted in southern Paraná (Feksa et al., 2019). However, our estimates were higher than those reported in the United States (41.8%) (Paul et al., 2018), which can be due to the higher number of sprays tested in our study. Furthermore, the estimates we reported for the other two QoI + DMI premixes (TFLX + PROT and TFLX + TEBU) were lower (<55%) than those from a previous study (>60%) that evaluated these premixes in curative (post-inoculation) sprays (Feksa et al., 2019). The premix containing QoI + DMI + SDHI (TFLX + PROT + BIXF) demonstrated poor performance (<50%) in controlling FHB. However, prior studies found improved performance for premixes amended with a novel SDHI called pydiflumetofen (Edwards 2022; Singh et al., 2021). Firstly, Edwards (2022) discovered that the co-formulation of pydiflumetofen and prothioconazole was more effective than either pydiflumetofen or prothioconazole alone. Secondly, a two-year study (2017 and 2019) in the U.S. observed a similar FHB severity reduction (~70%) for the premix of pydiflumetofen plus propiconazole, metconazole, and the premix of prothioconazole plus tebuconazole compared to the untreated check (Singh et al., 2021).

In our study, the most significant DON reduction (>58%) was achieved with metconazole alone and in combination with pyraclostrobin. However, Paul et al. (2018) reported a lower percent reduction of DON by METC (45%) and the premix of PYRA + METC (27%) after analyzing 292 uniform fungicide trials in the U.S. from 1995 to 2013. Similarly, a considerably lower DON reduction was reported for the premix of PYRA + METC (~30%) compared to METC (~90%) when applied alone in curative (post-inoculation) sprays (Feksa et al., 2019). Moreover, that study reported lower DON reduction (0–56%) for all treatments including a QoI (trifloxystrobin + prothioconazole, trifloxystrobin + tebuconazole, azoxystrobin + cyproconazole, and pyraclostrobin applied alone) (Feksa et al., 2019). On the contrary, the other premixes containing QoI + DMI in our study (TFLX + PROT and TEBU + TFLX) showed higher DON reduction (40–50%) compared to the previous study (Feksa et al., 2019). Despite the general recommendation against using QoIs for managing FHB, premixes of QoIs + DMIs have been employed by Brazilian growers to extend protection against foliar

Table 4
Unstandardized difference in wheat grain yield between fungicide-treated and untreated plots provided by six fungicides evaluated in 19 independent trials conducted across two Brazilian states (PR and RS) during 5 growing seasons (2017–2021).

Fungicide[a]	*k*[b]	Yield Response (kg/ha)				
		$\bar{D}$	SE ($\bar{D}$)	CI_L[c]	CI_U[c]	*P value*
TFLX + PROT + BIXF	13	643.8	70.1	506.2	781.3	<0.0001
METC	13	505.9	75.7	357.4	654.3	<0.0001
PYRA + METC	17	477.8	85.2	310.7	644.9	<0.0001
TFLX + PROT	13	455.3	80.4	297.6	613.0	<0.0001
CARB	17	453.2	75.6	304.9	601.4	<0.0001
TEBU + TFLX	17	403.4	55.7	294.1	512.7	<0.0001

[a] See Table 1 for complete information of the evaluated fungicides.
[b] Number of trials that each fungicide was evaluated.
[c] Upper (CI_U) and lower (CI_L) limits of the 95% confidence interval around $\bar{D}$.

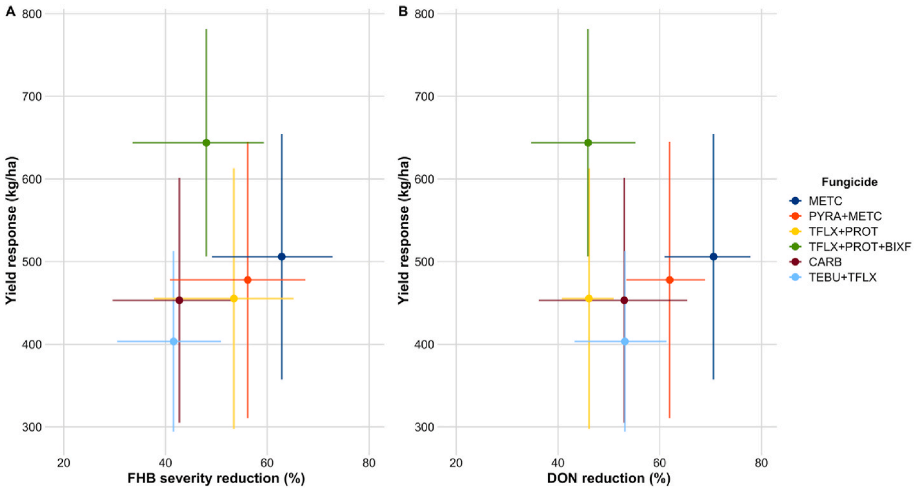

Fig. 2. Relationship between percent reduction of Fusarium head blight (FHB) (**A**), and between DON reduction and yield response relative to untreated check (**B**), for six fungicides evaluated across 19 independent field trials in Brazil from 2017 to 2021. Bars show the upper and lower limits of 95% confidence intervals around point estimates for both responses. See Table 1 for complete information of the evaluated fungicides.

diseases such as powdery mildew, tan spot, and rusts (Barro et al., 2017; Blandino et al., 2006; Paul et al., 2018; Ransom and McMullen 2008; Willyerd et al., 2012), resulting in improved yields, particularly in disease-favorable environments (Blandino et al., 2011; Wegulo et al., 2011).

Concerning the use of SDHIs, although the triple premix TFLX + PROT + BIXF reported a lower DON reduction (<40%), the premix of pydiflumetofen plus propiconazole reduced DON concentration by 52–73% compared to the untreated control (Xia et al., 2021). Additionally, significant DON reduction (~50%) was observed for the premix of pydiflumetofen plus propiconazole, metconazole, and the premix of prothioconazole plus tebuconazole compared to the untreated check in one year of a two-year study (2017 and 2019) in the U.S. (Singh et al., 2021).

The triple premix TFLX + PROT + BIXF yielded the highest wheat grain output (643 kg/ha). Despite that information on the presence and intensity of foliar diseases was not available in the primary studies used in our analysis, yield benefits from the use of DMI plus QoI and, more recently, SDHI premixes on wheat grain yields have been linked to a broad spectrum of protection, owing to the different modes of action, that could be extended to foliar diseases (Blandino et al., 2011; Bolanos-Carriel et al., 2020; Spolti et al., 2013; Wegulo et al., 2011). Blandino et al. (2006), for example, reported a 8.7% increase in yield by applying azoxystrobin + tebuconazole compared to the DMIs applied alone tebuconazole and prochloraz. Additionally, regarding physiological effects, wheat plants had a higher net CO_2 assimilation rate when treated with bixafen compared to a non-treated control (Berdugo et al., 2012). The response reported here for METC (505.9 kg/ha) is similar to the reports for metconazole applied once in the U.S. study, which provided the highest yield response (536 kg/ha) in spring wheat (Paul et al., 2010). Additionally, the yield estimates reported for PYRA + METC in the U.S. (435 kg/ha) (Paul et al., 2018) were slightly lower to the estimates reported here for the same premix (477 kg/ha). On the other hand, the yield response estimates for CARB reported in this study (453 kg/ha), were very similar to those from a previous meta-analysis (455 kg/ha on average) that assessed data from 2000 to 2015 (Machado et al., 2017).

To summarize, our study provides essential knowledge that can support informed decisions about the selection of fungicides to tackle FHB. It's important to evaluate not just technical factors such as effectiveness and the enhancement of yield, but also the reduction of DON levels. Regrettably, there are only a few studies which have documented the influence of fungicide application during flowering on DON levels (Bonfada et al., 2019; Feksa et al., 2019; Spolti et al., 2013), limiting our capacity to reliably estimate mycotoxin reduction as done in other research (Paul et al., 2018). It is of great importance to persist with the assessment of fungicides across various CFTs, and this effort should be encouraged. Furthermore, the conclusions from our study can be used as a guide when choosing fungicides for future investigations.

Author's contribution

JPB carried out the data analysis and wrote the manuscript. FMS also wrote the manuscript and oversaw the cooperative fungicide trials (CFTs) and shared the relevant data, while CST contributed to the writing and shared mycotoxin data. EMD offered insights on data analysis and writing. All other authors conducted the field experiments, and reviewed and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data and R codes used in the analysis are available as a research compendium in an OSF repository: https://doi.org/10.17605/OSF.IO/XQHPB

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2023.106402.

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