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Fungicide systemicity and blast control in wheat heads

Sistemicidade de fungicidas e controle da brusone em espigas de trigo

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Highlights _

All fungicides reduced blast severity compared to the control (water).

Systemicity differences were observed 5 and 7 days after inoculation.

Cyproconazole, Flutriafol, Propiconazole, Pydiflumetofen and Tebuconazole stood out.

Abstract _

The chemical control of wheat blast, a disease caused by *Pyricularia oryzae* Triticum (PoT), is part of the integrated management of this disease. The aim of this study was to evaluate fungicide systemicity in wheat heads and its effect on blast control. BRS 264 cultivars were grown in pots until anthesis (Zadoks stage 65), after which two central spikelets were removed from each head, exposing the rachis, where fungicide solutions were applied. Seven fungicides were evaluated (Flutriafol, Tebuconazole, Propiconazole, Pydiflumetofen, Cyproconazole, Pyraclostrobin and Mancozeb), as well as the control treatment with no fungicides and only water applied. Twenty-four hours after the solution was applied, a conidial suspension (10⁵ conidia mL⁻¹) of PoT was sprayed on the heads and the plants were kept at 24 °C, 90-95% relative humidity (RH) and in the dark for a further 24 hours. They were then kept at 24 °C, 70-80% RH under a 12-hour photoperiod until disease severity on the heads was assessed 5, 7 and 11 days after inoculation (dai). Between 20 and 30 heads were evaluated per treatment. Severity above and below the treated area was assessed by the generalized beta regression model. Above the treated area, at 5 and 7 days, the treatments with Flutriafol, Tebuconazole, Propiconazole, Pydiflumetofen and Cyproconazole differed from Check, showing lower average blast severity. Mancozeb and Pyraclostrobin also showed lower disease severity compared to the Check treatment when the overall results were

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analyzed. The acropetal movement of fungicides should be considered along with other approaches to integrated wheat blast management.

Key words: Pyricularia oryzae. Triticum aestivum. Chemical severity control.

Resumo .

O controle químico da brusone do trigo, uma doença causada por Pyricularia oryzae Triticum (PoT), faz parte do manejo integrado dessa doença. O objetivo deste estudo foi avaliar a sistemicidade dos fungicidas nas espigas de trigo e seus efeitos no controle da brusone. Plantas da cultivar BRS 264 foram cultivadas em vasos até a antese (estádio 65 de Zadoks), quando foram retiradas duas espiguetas centrais de cada espiga dessas plantas, expondo essa região da ráquis, onde foram aplicadas soluções fungicidas com pincéis. Foram avaliados sete fungicidas (Flutriafol, Tebuconazol, Propiconazol, Pidiflumetofen, Ciproconazol, Piraclostrobina e Mancozebe), além do tratamento de Controle, sem fungicida, aplicandose apenas água. Após 24 h da aplicação da solução, uma suspensão conidial (105 conídios mL-1) de PoT foi pulverizada nas espigas e as plantas foram mantidas a 24 °C, umidade relativa (UR) de 90-95% e no escuro por mais 24 h. Em seguida, permaneceram a 24 °C, UR de 70 a 80% e fotoperíodo de 12 h até que a severidade da doença nas espigas fosse avaliada aos 5, 7 e 11 dias após a inoculação (dai). Foram avaliadas entre 20 e 30 espigas por tratamento. Os dados de severidade acima e severidade abaixo da área tratada foram avaliados por generalized beta regression model. Acima da área tratada, aos 5 e 7 dai, os tratamentos com os fungicidas Flutriafol, Tebuconazol, Propiconazol, Pidiflumetofen e Ciproconazol diferiram do Controle, apresentando menor severidade média de brusone. Mancozebe e Piraclostrobina também apresentaram menor severidade da doença em comparação ao tratamento Controle quando analisados os resultados globais. O movimento acropetal dos fungicidas deve ser considerado junto de outras abordagens para o manejo integrado da brusone do trigo.

Palavras-chave: Pyricularia oryzae. Triticum aestivum. Severidade. Controle químico.

Introduction

The chemical control of wheat blast, a disease caused by the phytopathogen *Pyricularia oryzae* Triticum(PoT), an anamorph stage of *Magnaporthe oryzae* (Couch & Kohn, 2002), includes 70 fungicides currently registered in Brazil for aerial application, available on the market individually or in a mixture (Sistema de Agrotóxicos Fitossanitários [AGROFIT], 2024). These so-called site-specific fungicides can potentially inhibit demethylation activity (demethylation inhibitors - DMIs, known as

"triazoles") and mitochondrial respiration (succinate dehydrogenase inhibitors - SDHs and external quinone inhibitors - Qols, also called "carboxamides" and "strobilurins", respectively) (Amaro et al., 2020; Vicentini et al., 2021; Fungicide Resistance Action Committee [FRAC], 2024). In addition, the multi-site fungicide "mancozeb" is also widely recommended to control wheat blast (C. D. Cruz et al., 2019). However, available fungicides have difficulty controlling blast in wheat heads (Rocha et al., 2014; Santana et al., 2013), especially in crops with favorable environmental conditions, such as rainfall



during heading (C. D. Cruz et al., 2019). In this respect, there are reports of wheat fields with 100% losses due to blast damage (Kohli et al., 2011).

An important performance characteristic of fungicides is systemicity, that is, their ability to move within the plant. Thus, fungicides have acropetal and basipetal mobility (in an upward and downward systemic direction, respectively). Cyproconazole, tebuconazole epoxiconazole are examples of fungicides with acropetal movement (Liao et al., 2023). On the other hand, the number of fungicides with basipetal movement is limited (Liu et al., 2018; Oliver & Beckerman, 2022). Fungicides belonging to the strobilurin chemical group have a high affinity for the plant cuticle and are known to move mesostemically as they penetrate and move through the mesophyll (Bartlett et al., 2002).

The positioning of the spikelets around the rachis on the wheat heads protects this structure from being completely covered by fungicides during conventional field spraying (Sussel et al., 2021). In this scenario, the high systemicity of the fungicide is desirable for effective blast control, since fungicides that demonstrate mobility from their point of deposition on the plant, especially on the heads, can increase the effectiveness of disease control in the field.

Thus, considering the fundamental role of chemical control in wheat shoots in blast management (C. D. Cruz et al., 2019), it is essential to understand the dynamics of the systemicity of different fungicides for the *P. oryzae* Triticum x *Triticum aestivum* pathosystem in wheat head rachis, information that remains largely unknown. As such, this study aimed to evaluate fungicide systemicity in wheat heads and its effect on blast control.

Material and Methods ____

The study consisted of single experiment repeated once at different times in 2022, conducted in a controlled environment (greenhouse, inoculation chamber and laboratory) at Embrapa Trigo, Passo Fundo, Rio Grande do Sul state (RS), Brazil.

Growing plants and preparing heads

The experiments were carried out in a completely randomized design with eight treatments, consisting of seven fungicides and the Check treatment (T8) (Table 1). The latter did not receive any active ingredient with fungicidal action, only water. The Mancozeb treatment (T7) was used as a positive control due to its immobility (Reis et al., 2016). All the fungicides selected are composed of isolated active ingredients recommended for wheat blast control (AGROFIT, 2024).



Table 1
Characteristics of the fungicides used in the experiments

Treatments	Active ingredient (AI)	Chemical group	Commercial name	Formulation ¹	Al concentration²
T1	Flutriafol	Triazol	Tenaz	SC	0.25 g mL ⁻¹
T2	Tebuconazole	Triazol	UPL de Tebufort	CE	0.20 g mL ⁻¹
Т3	Propiconazole	Triazol	Propiconazol Nortox	CE	0.25 g mL ⁻¹
T4	Pydiflumetofen	Carboxamida	Miravis	SC	0.20 g mL ⁻¹
T5	Cyproconazole	Triazol	Alto® 100	SL	0. 10 g mL ⁻¹
T6	Pyraclostrobin	Estrobilurina	Comet	CE	0.25 g mL ⁻¹
T7	Mancozeb	Ditiocarbamato	Unizeb Gold	WG	0.75 g g ⁻¹
Т8	Check (water)	-	-	-	-

¹Formulation: SC: Suspension concentrate; EC: Emulsifiable concentrate; SL: Soluble liquid concentrate; WG: Water dispersible granule; ²Al: active ingredient.

The BRS 264 cultivar, which is susceptible to blast (Maciel et al., 2022; Cunha & Caierão, 2023), was sown in 8 L plastic pots. Ten plants were grown in each pot, which received the recommended wheat growing practices (Cunha & Caierão, 2023). For each treatment, we used wheat heads from four pots, evaluating between 20 and 30 heads per treatment.

The heads were prepared to receive the treatments when the wheat plants were at anthesis stage 65 on the Zadoks scale (Zadoks et al., 1974). On the first day, the heads were selected and marked; the central

spikelets (one on each side) were removed and the fungicide application area marked with a permanent ink pen (Figure 1A).

On the second day, the fungicide solutions corresponding to each treatment were prepared in a volume of 100 mL⁻¹, with fungicide doses established for wheat blast control under field conditions, according to the manufacturer's instructions. Using a soft bristle brush, the solutions were applied to the area marked on the rachis for each treatment (Figure 1B). To standardize application, only one coat of solution was applied to each side of the rachis.



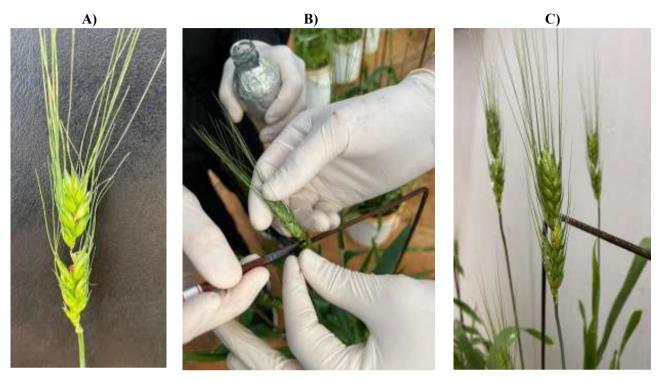


Figure 1. Wheat head preparation by removing two central spikelets (one on each side) and marking the area for fungicide application (A); fungicide solution application in the marked area (B); heads after inoculum spraying, carried out 1 day after fungicide application (C).

Preparing the inoculum

The conidia suspension was prepared as a homogeneous mixture of conidia from three PoT isolates (Py 15.1.010: Uberaba, Minas Gerais; Py 17.1.001: Passo Fundo, Rio Grande do Sul; Py 17.1.008: Sacramento, Minas Gerais). The isolates were classified into different virulence groups, according to the classification established by Pizolotto (2019) based on the reaction on the heads of 11 wheat and one barley genotype. The three PoT isolates belonging to the Embrapa Trigo reference collection were preserved at 4°C using the silica gel preservation method described by Perkins (1962).

The isolates were transferred individually to Petri dishes containing oatmeal agar culture medium (60 g.L⁻¹) and incubated for seven days in a 12-hour photoperiod at 25 °C for growth. The mycelium disks were then removed from the edge of the colony and re-deposited on Petri dishes with oatmeal agar culture medium. The plates were kept under the same conditions for 12 days.

To prepare the inoculum, the plates containing the PoT colonies were washed by adding distilled water with adhesive spreader (0.01% Tween 80). The conidia were dislodged with a glass slide and the solution filtered through a sieve with gauze. An aliquot was then collected to count the number of



conidia in a Neubauer chamber under an optical microscope. The concentration of the conidial suspension of each isolate was adjusted to 10⁵ conidia mL⁻¹. The suspensions of the three PoT isolates were mixed in equal volumes to make up the inoculum. The suspension of the conidial mixture was placed in a manual atomizer with a volumetric capacity of 0.5 L⁻¹ for inoculation.

Wet inoculation chamber

Twenty-four hours after the fungicide treatments were applied, the PoT inoculum was sprayed onto the wheat heads, with one spray above and one below the fungicidetreated area, and repeated on the other side of the head, for a total of four sprays of inoculum per head (Figure 1C). In the Check treatment, the heads received distilled water. The plants were then protected in a plastic humidity chamber, and kept in the dark for 24 hours, at 24°C and 90 to 95% relative humidity (RH). Next, the photoperiod and RH were adjusted to 12 h and 70-80%, respectively, maintaining these conditions for 11 days, coinciding with the latest assessment of blast symptoms on the wheat heads.

Statistical evaluations and analyses

To assess the treatments, each head was evaluated separately. Blast severity was assigned as a percentage 5, 7 and 11 days after inoculation (dai), in two regions: above (severity above) and below the treated area (severity below).

Severity data (severity above and severity below) were assessed using a generalized beta regression model (GZLM) (Ferrari & Cribari, 2010), applying logit as the link function. The model's eligibility criteria were assessed and met. To ensure a suitable beta regression model, values equal to 0 or 1 were adjusted to 0.001 and 0.999, respectively, to preserve proportionality. The treatments and dai (5, 7 and 11) were considered a fixed effect and the pots a random effect. In addition, we evaluated time (5, 7 and 11 days) as a random effect, keeping treatments as a fixed effect.

The analysis included 192 observations and was conducted in stages, starting with descriptive statistics of the variables of interest, including the mean and standard deviations of the severity proportions for the treatments and times evaluated, as well as graphs of the confidence interval of the mean. The models were fit using the maximum likelihood method, applying confidence intervals calculated by the Wald method.

Omnibus tests were carried out to assess the overall significance of the main effects of time and treatment, as well as the interaction between them, and the statistical significance (p value) was reported. Tukey's post hoc test was used for multiple comparisons and the results included mean differences between groups or periods and their respective p values. The analyses were conducted using Jamovi project (2024) software, considering a significance level of 0.05.



Results _

Severity above (%) data

Table 2 shows the main fit indices of the GZLM model for blast severity above the fungicide application area. The pseudo-R² coefficient of determination was 0.91 with p < 0.001, indicating that 91% of the variance in severity above was explained by the variables included in the model. The Bayesian information criterion (BIC) was -548.48 and deviance 440.42, indicating a good overall fit. The ratio between the chi-square and

the residual degrees of freedom was 1.17, indicating an adequate fit since it was close to 1.0.

The global omnibus tests revealed that there was a significant treatment effect on severity above (p < 0.001), and a significant effect of time (p < 0.001), indicating statistically significant changes over time. However, the interaction between treatments and time was not significant (p = 0.235), demonstrating that the change in blast severity over time was consistent between treatments.

Table 2
Model adjustment variables for blast severity (%) above the fungicide application area

Indexes	Result
R ² (p)	0.91
p value	< 0.001
BIC	-548.48
Deviance	440.42
Residual DF	167
Chi-squared/DF*	1.17

^{*} residual degrees of freedom.

Considering the whole period, we found a significant difference between the treatment groups (p < 0.001) (Table 3). When analyzing the time factor separately, we found significant intergroup differences at 5 (p < 0.001) and 7 days (p = 0.002), but not at 11 days (p = 0.856). The intra-group p-values indicated significant changes in severity

above over time (p < 0.001) for all treatments. Table 4 shows the comparison of severity below data between the three evaluations. All comparisons between days were statistically significant (p < 0.001), indicating that severity above increased progressively over time after inoculation.



Table 3
Descriptive statistics of blast severity (%) above the fungicide application area showing comparison by treatment and time (days after inoculation)

Severity above	All period		Days after inoculation						p value
(%)			5		-	7			intragroup**
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Check	77.95	26.85	50.26	18.63	83.58	23.52	100.00	0.00	<0.001
Cyproconazole	60.70	36.39	24.95	18.72	57.16	27.57	100.00	0.00	<0.001
Flutriafol	59.90	34.91	19.38	6.08	60.41	16.12	99.92	0.22	<0.001
Mancozeb	69.32	32.05	31.29	16.29	76.65	17.90	100.00	0.00	<0.001
Propiconazole	60.91	33.99	21.61	6.92	61.11	15.38	100.00	0.00	<0.001
Pydiflumetofen	61.94	35.68	20.46	11.42	65.38	20.42	100.00	0.00	<0.001
Pyraclostrobin	69.79	31.07	35.60	20.24	73.84	19.22	99.94	0.18	<0.001
Tebuconazole	57.83	38.00	16.64	7.22	56.89	26.91	99.96	0.11	<0.001
p value between groups*	<0.001		<0.001		0.002		0.856		

¹SD: standard deviation; * Beta Generalized Linear Model (GZLM).

Table 4
Post hoc test comparing overall blast severity (%) above the fungicide application area between times (days after inoculation - dai)

Time	vs	Time	Difference	p value*
5dai	-	7dai	-0.40	< 0.001
5dai	-	11dai	-0.68	< 0.001
7dai	-	11dai	-0.28	< 0.001

^{*} Tukey at 0.05 significance level.



In severity above analysis considering the whole period (Table 5), i.e. without the influence of the time factor (days), we observed higher values, with a significant difference between the Check treatment and Cyproconazole, Flutriafol, Propiconazole, Pydiflumetofen and Tebuconazole (p < 0.001), as well as Pyraclostrobin (p = 0.011) and Mancozeb (p = 0.026). None of the comparisons between the other treatments showed statistically significant differences (p > 0.05).

Table 5
Post hoc test comparing the overall blast severity (%) above the fungicide application area between treatments

Treatment	vs	Treatment	Difference	p value*
Check	-	Cyproconazole	0.18	<0.001
Check	-	Flutriafol	0.19	<0.001
Check	-	Mancozeb	0.10	0.026
Check	-	Propiconazole	0.18	<0.001
Check	-	Pydiflumetofen	0.17	<0.001
Check	-	Pyraclostrobin	0.11	0.011
Check	-	Tebuconazole	0.19	<0.001

^{*} Tukey at 0.05 significance level.

According to Table 6 and Figure 2, the Check treatment demonstrated significantly higher severity above values compared to the Cyproconazole, Flutriafol, Propiconazole, Pydiflumetofen and Tebuconazole treatments at 5 and 7 dai (p < 0.05). We observed no significant differences

between Check and Mancozeb (p > 0.05) or between Check and Pyraclostrobin (p > 0.05) (Table 6), according Figure 3. In addition, no comparison of severity above between times and treatments was significant at 11 dai (p > 0.05), indicating no differences in control between treatments in this evaluation.



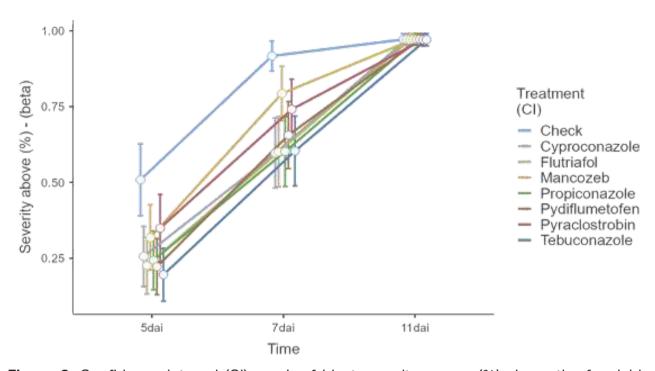


Figure 2. Confidence interval (CI) graph of blast severity means (%) above the fungicide application area by treatment and time (days after inoculation – dai).

Table 6
Post hoc test comparing the overall blast severity (%) above the fungicide application area between time (days after inoculation - dai) and treatments

Treatment	vs	Time	Treatment	Difference	p value*
Check	-	5dai	Cyproconazole	0.26	0.002
Check	-	5dai	Flutriafol	0.29	<0.001
Check	-	5dai	Mancozeb	0.20	0.078
Check	-	5dai	Propiconazole	0.28	<0.001
Check	-	5dai	Pydiflumetofen	0.30	<0.001
Check	-	5dai	Pyraclostrobin	0.17	0.257
Check	-	5dai	Tebuconazole	0.33	<0.001
Check	-	7dai	Cyproconazole	0.30	0.005
Check	-	7dai	Flutriafol	0.30	0.005
Check	-	7dai	Mancozeb	0.12	0.601
Check	-	7dai	Propiconazole	0.30	0.006
Check	-	7dai	Pydiflumetofen	0.25	0.032
Check	-	7dai	Pyraclostrobin	0.17	0.258
Check	-	7dai	Tebuconazole	0.30	0.006
	Check	Check -	Check - 5dai Check - 7dai	Check - 5dai Cyproconazole Check - 5dai Flutriafol Check - 5dai Mancozeb Check - 5dai Propiconazole Check - 5dai Pydiflumetofen Check - 5dai Pyraclostrobin Check - 5dai Tebuconazole Check - 7dai Cyproconazole Check - 7dai Flutriafol Check - 7dai Mancozeb Check - 7dai Propiconazole Check - 7dai Propiconazole Check - 7dai Pydiflumetofen Check - 7dai Pydiflumetofen Check - 7dai Pyraclostrobin	Check - 5dai Cyproconazole 0.26 Check - 5dai Flutriafol 0.29 Check - 5dai Mancozeb 0.20 Check - 5dai Propiconazole 0.28 Check - 5dai Pydiflumetofen 0.30 Check - 5dai Pyraclostrobin 0.17 Check - 5dai Tebuconazole 0.33 Check - 7dai Cyproconazole 0.30 Check - 7dai Flutriafol 0.30 Check - 7dai Mancozeb 0.12 Check - 7dai Propiconazole 0.30 Check - 7dai Propiconazole 0.30 Check - 7dai Pydiflumetofen 0.25 Check - 7dai Pyraclostrobin 0.17

^{*} Tukey at 0.05 significance level.



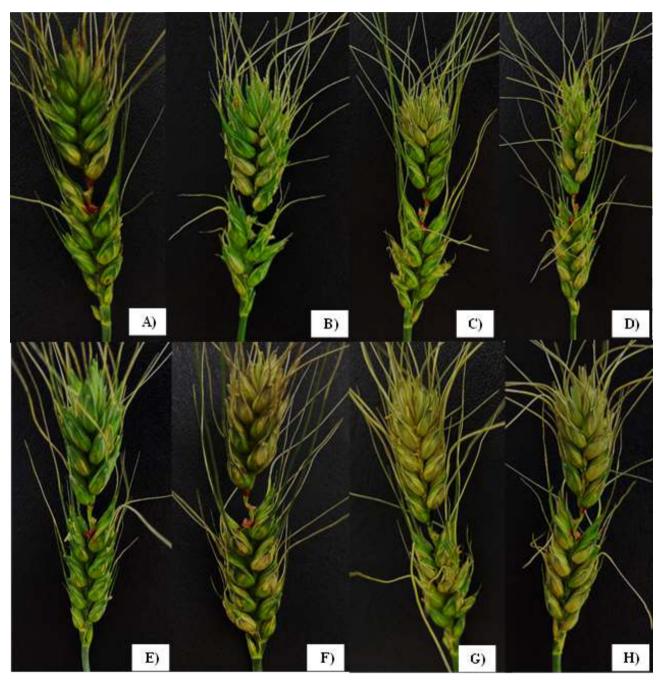


Figure 3. Blast symptoms above and below fungicide application to wheat heads: Flutriafol (A), Tebuconazole (B), Propiconazole (C), Pydiflumetofen (D), Cyproconazole (E), Pyraclostrobin (F), Mancozeb (G) and Check (water). BRS 264 cultivar, 5 days after inoculation and six days after fungicide application.



Severity below (%) data

The main model fit indices for blast severity below the fungicide application area are shown in Table 7. The pseudo-R² coefficient of determination was 0.80 with p < 0.001, indicating that 80% of the variance in severity below was explained by the variables included in the model. The Bayesian information criterion (BIC) was -432.43 and deviance 408.28, demonstrating a good overall fit. The ratio between the chi-square

and the residual degrees of freedom was 0.96, indicating an adequate fit.

The omnibus tests revealed that there was no significant treatment effect on severity below (p = 0.585). On the other hand, a significant effect of time was identified (p < 0.001), demonstrating significant changes in severity over time. The interaction between treatment and time was not significant (p = 0.977), indicating that the effect of time was consistent regardless of the treatment used.

Table 7
Model adjustment variables for blast severity (%) below the fungicide application area

Indexes	Result
R ² (p)	0.80
p value	<0.001
BIC	-432.43
Deviance	408.28
Residual DF	167
Chi-squared/DF*	0.96

^{*} residual degrees of freedom.

We also found no significant difference between the treatments for severity below when considering the whole period (p = 0.995) (Table 8). Likewise, as shown in Figure 4, analysis of the differences between treatments at 5, 7 and 11 days were not statistically significant (p > 0.05). The intra-group p-values indicated that there were significant changes in severity below

over the days evaluated for all treatments (p < 0.001). Table 9 shows the comparison of the severity below data between the three evaluation dates. All comparisons were statistically significant (p < 0.001). Thus, as with severity above, severity below increased progressively between 5, 7 and 11 dai in all treatments.



Table 8
Descriptive statistics of blast severity (%) below the fungicide application area showing comparison by treatment and time (days after inoculation)

Severity below	All period		Days after inoculation						p value
(%)			5		7		11		intragroup**
Treatment	Mean	SD ¹	Mean	SD	Mean	SD	Mean	SD	
Check	60.94	39.73	15.42	5.45	69.65	33.70	97.74	4.22	<0.001
Cyproconazole	53.57	38.44	10.48	4.01	53.63	23.70	96.59	6.25	<0.001
Flutriafol	56.44	41.16	11.18	4.26	60.48	35.03	97.67	4.32	<0.001
Mancozeb	58.56	39.01	12.57	5.59	65.85	27.36	97.25	5.59	<0.001
Propiconazole	59.32	38.15	15.55	6.45	63.01	26.36	99.41	1.50	<0.001
Pydiflumetofen	56.66	41.23	9.30	3.78	61.24	29.83	99.44	1.59	<0.001
Pyraclostrobin	63.37	37.49	16.74	4.10	73.51	21.82	99.88	0.35	<0.001
Tebuconazole	55.65	40.69	11.52	3.74	56.10	31.93	99.33	1.24	<0.001
p value between groups*	0.995		0.090		0.768		0.995		

¹SD: standard deviation; * Beta Generalized Linear Model (GZLM).

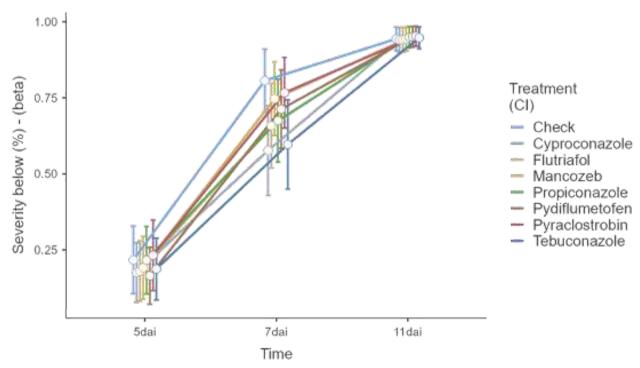


Figure 4. Confidence interval (CI) graph of blast severity (%) means below the fungicide application area by treatment and time (days after inoculation – dai).



Table 9
Post hoc test comparing overall blast severity (%) below the fungicide application area between days after inoculation (dai)

Time	vs	Time	Difference	p value*
5dai	-	7dai	-0.50	<0.001
5dai	-	11dai	-0.75	<0.001
7dai	-	11dai	-0.25	<0.001

^{*} Tukey at 0.05 significance level.

No statistically significant differences were observed in the severity below data between the different treatments or between different times and treatments.

Discussion

To the best of our knowledge, this is the first study to show the effect of fungicide systemicity in wheat head rachis on blast control. In addition, we show significant differences in terms of fungicide systemicity, through acropetal movement in wheat heads of the main active ingredients with fungicidal action used to control wheat blast in Brazil, compared to the treatment without fungicide (Check) (Table 3, Figure 3). In this respect, it is important to underscore the potential for superior disease control under field conditions using the fungicides Cyproconazole, Flutriafol, Propiconazole, Pydiflumetofen and Tebuconazole (Table 2). However, it is important to note that heads treated with Mancozeb and Pyraclostrobin also exhibited lower brusone severity when data analysis was conducted globally, without considering the assessment days separately.

Fungicide performance, compared to the Check treatment, and 7 days after inoculation, was essential in identifying treatments with systemic activity in wheat heads. In relation to systemicity criteria and consequent blast control, the systemicity classification is another aspect to be considered when choosing the fungicide to be applied to wheat crops. With respect to the applicability of this information, field tests are highly recommended to validate the results obtained in our study. Although the effectiveness of applying fungicides to control wheat blast is highly questionable (C. D. Cruz et al., 2019; Maciel, 2011), the fungicides that differed from Check in our study may exhibit better blast control under field conditions and where the disease occurs naturally. This is based on the basic principle associated with fungicide systemicity in a plant in the field, a characteristic that should be considered an important technical advantage. Such a fungicide has a greater capacity to reach wheat head areas, such as the rachis and regions below the spikelets, where droplets generated during spraying generally cannot reach.



In regard to blast progression in the experiments, we found that the disease reached its highest values at 11 days (Tables 3 and 8), with averages close to 100% severity, and no differences between the treatments when compared to the treatment without fungicide. This behavior requires a detailed analysis. A simplified analysis suggests that there is no technical advantage in using systemic fungicide, given that the disease has progressed and no significant differences were observed between the treatments at 11 days. In contrast to this analysis, we observed that until the end of the evaluations, highly favorable environmental conditions were maintained for the development of the disease. Thus, we emphasize that blast had no problem developing in the trials, since the plants were kept at 24°C and RH between 70 and 80% until 11 dai, ideal conditions for wheat head infection and colonization by PoT. On the other hand, in a field environment, several factors can hinder disease progression, allowing, for example, the combined action of a systemic fungicide with a contact fungicide to inhibit germination, infection and colonization of the pathogen. Among these factors, the environmental conditions favorable to blast in wheat fields tend to be short-lived, in contrast to the controlled environment of the experiment, where ideal conditions for blast development were intentionally provided. Another example of these factors is the use of blast-resistant wheat cultivars which, when combined with the use of fungicides, can limit development of the disease. In our experiments, the high susceptibility of BRS 264 to blast (Maciel et al., 2022; Albrecht, 2021) contributed to the high disease severities observed even with fungicides (Table 3).

The results of the evaluations, which showed blast control above the fungicide application point (Tables 3 and 5; Figure 3), corroborate the importance of the acropetal movement of these products in the chemical control of blast. In addition, observing the development of disease symptoms in wheat heads contributes to understanding the dynamics of infection by the pathogen. We detected PoT infection by the characteristic "blanching" of the head, beginning at the point of penetration of the pathogen into the rachis (Lau et al., 2020). This symptom occurs due to obstruction of the conducting vessels in the rachis and does not necessarily reflect an active infectious process in the upper portions of the wheat head. Four days after infection, PoT hyphae extensively colonize all the rachis tissues, including the epidermis, collenchyma, cortical parenchyma, pith and vascular bundle (M. F. A. D. Cruz et al., 2015). Thus, in some treatments in our study, infections below the fungicide application areas may have produced symptoms in the upper part of the heads, above the application area, raising the "severity above" values. This occurred to a greater or lesser degree, depending on the systemic capacity of the fungicide used.

Analysis of the severity above data (Table 6) revealed no significant differences between the Mancozeb and Pyraclostrobin treatments and the Check treatment, results that were expected and which validate the methodology used to assess fungicide systemicity. The lack of difference can be explained by the characteristics of these active ingredients, Mancozeb immobility (Reis et al., 2016) and the translaminar activity of Pyraclostrobin (Bartlett et al., 2002), attributes that compromise



the ability of these active ingredients to significantly reduce blast severity. Although our results show greater blast severity in areas not directly treated with Mancozeb or Pyraclostrobin, this does not invalidate the recommendation of products based on these active ingredients for controlling the disease in wheat. It is important to emphasize that, when analyzing the overall results, regardless of the time factor (dai), both treatments showed some level of control, resulting in significant differences in relation to the fungicide-free Check treatment (Table 5). It is important to consider that Mancozeb is widely recommended in association with site-specific fungicides, since it offers multi-site action against pathogens, making it crucial for anti-resistance management (FRAC, 2024). Studies by the cooperative trial network, called Network of Cooperative Trials for Resistance to Wheat Blast on Heads (RECORBE), have shown a reduction in grain yield losses using Mancozeb (Mancozeb, Mancozeb + Azoxystrobin, Mancozeb + Thiophanate-methyl) (Cunha & Caierão, 2023).

We believe that the unsatisfactory results in attempts to chemically control wheat blast in wheat crops is strongly related to the inherent architecture of wheat heads in the crop canopy. Wheat heads are arranged vertically, in a cylindrical shape, with the apex exposed to the spray jet, unlike what occurs with leaves. During crop spraying, wheat heads are a difficult target to reach due to this characteristic, resulting in uneven fungicide spray distribution, especially on the rachis, where only 8.54% of all the product sprayed to control wheat blast on the head reaches this structure (Sussel & Zacaroni, 2021). Given

these particularities, together with the fact that PoT infects all aerial organs of the wheat plant (Valent et al., 2021), it is essential to seek the greatest possible droplet coverage when applying fungicides, with an emphasis on protecting the heads. In this respect, although studies on fungicide application technology for controlling wheat blast are still incipient (Torres et al., 2022), factors such as the appropriate choice of spray tip play an essential role in fungicide distribution and effectiveness. Attention to these details can ensure better head protection and increase the effectiveness of wheat blast control in crops. In addition, the use of adjuvants, such as adhesive spreaders, can aid in fungicide applications (Aguiar et al., 2011), improving spray deposition and, consequently, wheat blast control (Pizolotto, 2019).

In light of the present study, it is also essential to recognize that, under crop conditions, fungicides are the last resort available during the harvest to minimize the impacts of wheat blast. In this scenario, identifying and incorporating sources of genetic resistance to wheat blast should be prioritized (Maciel et al., 2024). This approach not only complements chemical control practices but also contributes to safer wheat farming in regions susceptible to wheat blast epidemics.

Conclusions _

We demonstrated that the fungicides Cyproconazole, Flutriafol, Propiconazole, Pidiflumetofen and Tebuconazole exhibited lower blast severity and consequently greater systemicity, differing significantly from Check in the evaluations carried out



5 and 7 days after inoculation. In regard to fungicide use in wheat crops, we recommend applying these products to the shoots in an integrated management approach for wheat blast. In this sense, it is essential that the chosen fungicide(s) offer(s) broad coverage of the wheat head and significant advantages in relation to systemic capacity. This can be achieved by using systemic fungicides combined with multi-sites, which control the fungus at the onset of the infectious process. It is important to consider both aspects to protect the heads, given their direct economic importance for the wheat crop.

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