Fusarium Head Blight and Deoxynivalenol: What is the relationship?

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Abstract – Fusarium head blight (FHB), mainly caused by the fungus *Fusarium gramineraum*, is one of the most common wheat diseases. Besides causing yield reduction, it is also associated with the accumulation of mycotoxins in kernels, especially deoxynivalenol (DON). This study was performed during the 2019 and 2020 harvest seasons and aimed at using different sowing dates, cultivars, and fungicide applications to help to increase yield and kernel quality (decrease in DON contamination). This study was performed with a randomized block design. Weak correlations (R<0.50) between FHB severity, incidence of *Fusarium* in kernels, and DON accumulation were observed. Fungicides helped to increase yields and achieve better hectoliter weight and weight of a thousand kernels, mainly in 2019 (the most humid year). Meteorological conditions affect yields and kernel quality. The use of fungicides promotes a good disease control response and can be considered a good tool to help farmers to achieve greater yields. The best way to predict DON accumulation would be analyzing the incidence of *Fusarium* in kernels.

Index terms: DON; Fusarium graminearum; Mycotoxins; Wheat.

Giberela e Desoxinivalenol: Qual a relação?

Resumo – A Giberela, causada principalmente pelo fungo *Fusarium graminearum*, é uma das doenças mais comuns em trigo. Além de causar reduções na produtividade da cultura, também é responsável pelo acúmulo de micotoxinas, especialmente a desoxinivalenol (DON), em grãos de trigo. Este trabalho foi desenvolvido durante os anos agrícolas de 2019 e 2020, buscando avaliar a utilização de diferentes cultivares, diferentes datas de plantio e diferentes manejos com fungicidas para aumentar a produtividade e a qualidade dos grãos (menor incidência de DON). O trabalho foi conduzido sob esquema de blocos casualizados. Fracas correlações (R<0,50) entre a severidade da giberela, incidência de *Fusarium* nas sementes e acúmulo de DON foram observadas. O uso de fungicidas ajudou a aumentar a produtividade, o peso hectolitro e o peso de mil sementes do trigo, principalmente em 2019 (ano mais úmido). Condições meteorológicas afetam os rendimentos e a qualidade dos grãos. O uso de fungicidas exerce uma boa resposta no controle de doenças e pode ser considerado uma boa ferramenta para auxiliar agricultores a obter maiores produtividades. A melhor maneira de prever o acúmulo de DON seria analisando a incidência de *Fusarium* nos grãos.

Termos para indexação: DON; Fusarium graminearum; Micotoxinas; Trigo.

Introduction

Fusarium head blight (FHB) is a disease that affects cereal crops worldwide and causes significant losses to farmers and industries because of yield reduction and reduction in kernel quality. This disease is caused mainly by the fungus *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* [Schweinitz]Petch) and its incidence in regions where the no-tillage system is used tends to be much more frequent because of the presence of a greater inoculum (WEGULO, 2012).

Besides yield reduction, which was about 60% to 70% in South American fields (RANDHAWA et al., 2019) and 18.6% to 39.9% in Brazilian fields (REIS et al., 2016), FHB also decreases kernel quality due to mycotoxin biosynthesis and accumulation by F. graminearum (BONFADA et al., 2019), which may reduce the commercial value of the crop up to 100% in many cases (MARIN et al., 2013). McMullen et al. (2012), in a review focusing on FHB in barley and wheat, showed that the United States of America lost about US\$ 7.67 billion due to the incidence of this disease from 1993 to 2001.

However, the presence of mycotoxins in cereal kernels increased attention of governmental agencies worldwide for the consequences that the intake of exaggerated amounts of mycotoxins can cause to humans and livestock (MARIN et al., 2013). The most problematic and recurrent mycotoxin found in winter cereals, such as wheat and barley, is deoxynivalenol (DON), also known as vomitoxin. DON is a type B trichothecene that is linked to vomiting disorders, weight loss, diarrhea, neurological/immunological problems, and other health problems (PINTON & OSWALD, 2014).

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Mycotoxins (from the Greek words *mikes*, for "mold," and *toxicum*, for "poison") are considered the fungi venom and their biosynthesis is believed to be linked to fungi stress-response defense mechanisms against biotic and abiotic conditions (PONTS, 2015), including the use of fungicides.

Therefore, the use of inefficient fungicides to control specific fungi, such as F. graminearum, inefficient doses, and fungicides in inappropriate periods, besides the incidence of biotic and abiotic stresses, might contribute higher mycotoxin biosynthesis to and accumulation (WEGULO, 2012; BONFADA et al., 2019). This might be the reason why the correlation between the FHB incidence and DON in wheat and barley kernels tends to be weak (PONTE et al., 2012; WEGULO, 2012). Merhej et al. (2010) showed that Fusarium can be induced to synthetize more DON when stressed, varying the pH of the growth medium. It shows that similar amounts of Fusarium might synthetize different amounts of mycotoxins as a stressresponse mechanism.

Understanding the problematic consequences of the FHB incidence and DON in cereal kernels, this study presents data from a two-year research performed in Southern Brazil with different amounts of fungicides (applications), cultivars, and sowing dates, aiming to 1) reduce FHB severity, 2) reduce DON accumulation in wheat kernels, and 3) raise crop yield.

Material and Methods

This study was performed in Chapecó, SC, Brazil (altitude of 623m), during the 2019 and 2020 wheat harvest seasons. The study area is in a Cfa Köppen-Geiger climatic location (BECK et al., 2018) and presents a typical dystroferric red oxisol. BRS 374 (2019 and 2020) and ORS Agile (2020) were the wheat cultivars used. Both cultivars were sowed in a no-tillage field using 330 viable seeds m⁻². In both years, 300kg ha-1 08-30-20 N-P_O_-K_O fertilizer were applied in the area at sowing and 45kg ha-1 nitrogen were applied twice equally at the beginning and middle of the tillering stage.

The sowing was performed on two different dates in 2019 (June 28th and July 11th) and 2020 (June 16th and June 29th) in plots of $5 \times 1m$ (length \times width) and sowing lines spread 20cm apart from each other. Each plot was spread one meter apart from one another. Seed treatment was performed using the fungicide/nematicide Certeza® (0.2ml kg⁻¹ of seeds) and the insecticide Cruiser Opti[®] (0.25ml kg⁻¹ of seeds). The foliar disease control for the vegetative period was made during the stages of development: tillering (3.0), elongation (6.0), and booting (10.0), according to the Large's scale (1954). Fox XPro® (0.5l ha⁻¹) + Aureo[®] (0.25l ha⁻¹) were the fungicides used for stage 3.0, Ativum® (1.2l ha⁻¹) + Assist[®] (0.5l ha⁻¹) for stage 6.0; and Fox XPro[®] (0.5l ha⁻¹) + Aureo[®] (0.25l ha⁻¹) for stage 10.0 (Table 1).

During the flowering stage, three additional applications of fungicides performed, spread were seven days apart from each other, for the prevention/control of foliar/ear diseases, mainly FHB. These applications started in stage 10.5 (beginning of the flowering period). Bendazol[®] (0.6 l.ha⁻ ¹) was the fungicide used for the first application and Tebufort[®] (0.75L ha⁻¹) for the second and third applications (Table 1). All applications of fungicides (in the vegetative and reproductive stages) were performed using 250l ha⁻¹ of water

and a portable compressed CO₂ sprayer.

This study was performed under a randomized block design and composed of five treatments, two sowing dates, and four replicates. The treatments were: A (control: no fungicide application), B (fungicide application in stages 3.0, 6.0, and 10.0), C (fungicide application in stages 3.0, 6.0, and 10.0 and one additional application at the beginning of the flowering stage), D (fungicide application in stages 3.0, 6.0, 10.0 and two additional applications at the flowering stage), and E (fungicide application in stages 3.0, 6.0, and 10.0 and three additional applications at the flowering stage).

The severity of fusarium head blight (%) was evaluated every seven days from the beginning of the flowering stage until kernels reached their physiological maturity. For the evaluation of FHB severity, thirty randomly selected ears were analyzed and averaged according to the scale proposed by Stack & McMullen (2011). After harvest (on October 10th and November 11th 2019 and October 14th and 22nd 2020), all kernels were analyzed for their humidity (%) and the hectoliter weight (HW), weight of a thousand kernels (WTK), and yield (kg ha⁻¹) were adjusted to a 13% moisture. One hundred kernels of each plot were sent to the lab to be pathologically

Table 1. Commercial products used during experiments, their active ingredients and concentrations

Tabela 1. Produtos comerciais usados durante os experimentos, seus princípios ativos e
concentrações

Commercial product	Active ingredients
Certeza®	Thiophanate-methyl (350g.l ⁻¹), Fluazinam (52.5g.l ⁻¹)
Cruiser Opti [®]	Lambda-Cyhalothrin (37.5 g.l ⁻¹), Thiamethoxam (210 g.l ⁻¹), Petroleum Naphtha (34.6 g.l ⁻¹)
Fox XPro [®]	Bixafen (125g.l ⁻¹), Prothioconazole (175g.l ⁻¹), Trifloxystrobin (150g.l ⁻¹)
Ativum®	Epoxiconazole (50g.l ⁻¹), Fluxapyroxad (50g.l ⁻¹), Pyraclostrobin (81g.l ⁻¹)
Bendazol [®]	Carbendazim (500g.l ⁻¹)
Tebufort [®]	Tebuconazole (200g.l ⁻¹)
Assist [®]	Mineral oil (756g.l ⁻¹)
Aureo®	Soybean oil methyl ester (720g.l ⁻¹)

analyzed for the presence of F. araminearum. Kernels were disinfected with NaClO (50%), transferred to a germ-box with BDA + antibiotic medium and incubated for seven days at 25 ± 2°C and a 12-hour photoperiod. Kernels were considered infected when mold growth was observed. The incidence of F. graminearum was expressed in percentage (%) of kernels contaminated. The quantification of deoxynivalenol was performed using 100g of kernels from each plot by the high sensitivity test Enzyme-Linked Immunosorbent Assay (ELISA) from Neogen[®], Brazil (Veratox[®] HS - detection level of 25 ppb).

All data were assessed for their normality and the existence of outliers. When a normal distribution of the data was found, analysis of variance (ANOVA) was performed and their means, when significantly different, were compared by a Tukey HSD statistical test. When ANOVA assumptions were not satisfied, data were analyzed via Kruskal-Wallis and their means, when significantly different, were compared by the Mann-Whitney statistical test. Pearson correlations were performed between the variables "FHB severity × DON accumulation," "FHB severity × incidence of Fusarium in kernels," and "DON accumulation × incidence of Fusarium in kernels." A contrast analysis was performed comparing DON global accumulation in treatments B, C, D, and E with the control treatment (A), regardless of the year, sowing date, and cultivar. All statistical analyses were performed via SAS JMP® Pro 14.0.0 (RRID:SCR 014242).

Results and discussion

Table 2 presents the meteorologic data of the study area during the 2019 and 2020 harvest seasons. Gray-shaded cells represent the most critical period for FHB incidence and development in wheat ears, going from the anthesis until the soft-dough stage of kernels (WEGULO, 2012). Therefore, constant rainfalls during these months might have contributed to higher FHB infestations in wheat plants. For instance, the 2019 harvest season presented much more rainfall during the critical period for FHB incidence (465.6 mm) when compared with the 2020 harvest season (316.2 mm).

In the 2019 harvest season, yield was significantly different within each sowing date (p<0.0001), as well as between sowing dates (p<0.0001). Seeds sown earlier in 2019 generated plants with higher potential to achieve greater yields (Table 3). Moreover, in 2019, HW was significantly different within each sowing date (p<0.0001) and between sowing dates (p<0.0001). WTK was only significantly different within each sowing date (p<0.0001). In this same year, fungicides, regardless of the number of applications, increased yield, WTK, and HW.

On the other hand, in the 2020 harvest season, for both cultivars, yield, WTK and HW presented no significant differences within each sowing date, regardless of the treatment (Table 3). However, the cultivar BRS374 presented significant differences between sowing dates for the variables yield (p<0.0001), WTK (p<0.0001), and HW (p<0.0001). The cultivar ORS Agile presented significant differences only between sowing dates for the variables yield (p=0.0002) and WTK (p<0.0001), which shows again that earlier sowing allowed greater yields.

We performed non-parametric analyses for variables related to kernel quality, including DON accumulation (ppb), FHB severity (%), and incidence of *Fusarium* in kernels (Table 4). We only found significant difference within each sowing date for DON accumulation in 2020 for the cultivar ORS Agile at the earlier sowing date, which received three applications of fungicides in comparison with the control treatment (no fungicide application).

However, when comparing sowing dates for each year and cultivar (only regarding DON accumulation), the cultivar BRS 374 presented significant differences in 2019 (p<0.0001) and 2020 (p=0.0097).

Pearson correlations between the variables FHB severity (%), DON accumulation, and incidence of *Fusarium* in the kernel resulted in weak correlations (R=0.09 to 0.50). The strongest interaction was for the correlation between "incidence of *Fusarium* in the kernel × DON accumulation," resulting in R=0.5011 (Figure 1).

Interestingly, when we performed a

Table 2. Meteorologic data from June to November in the 2019 and 2020 harvest seasons - Chapecó, SC

Tabela 2. Dados meteorológicas entre os meses de jun	ho e novembro para as safras de
2019 e 2020 - Chapecó, SC	

		Jun.	Jul.	Aug.	Sept.	Oct.	Nov.
	Mean temperature (°C)	17.97	14.42	15.80	19.45	21.71	22.58
2019	Mean relative humidity (%)	71.55	72.07	66.16	63.21	68.23	69.18
	Accumulated precipitation (mm)	43.4	92.8	42.2	42.8	215.6	165
	Mean temperature (°C)	16.39	14.97	17.28	20.51	22.02	22.02
2020	Mean relative humidity (%)	78.50	73.61	65.61	65.61	59.91	58.78
	Accumulated precipitation (mm)	333	128.6	117.8	40.2	19.2	139

Gray-shaded cells show the critical period for FHB incidence in wheat, going from the anthesis until the soft-dough stage (WEGULO. 2012).

Table 3. Yield, weight of a thousand kernels (WTK), and hectoliter weight (HW) for the 2019 and 2020 harvest seasons of according to the sowing date, cultivar, and treatment. Chapecó, SC

Tabela 3. Produtividade, peso de mil sementes (PMS) e peso hectolitro (PH) para as safras 2019 e 2020 em função da época de plantio, da cultivar e tratamento. Chapecó, SC

Year	Cultivar	Sowing date	Treatment	Yield (kg.ha⁻¹)	WTK (g)	HW
			А	3577.14 bC	22.87 b ^{NS}	70.75 bE
			В	5131.18 aA	29.04 a	74.67 aABC
		June 28 th	С	5096.13 aA	30.61 a	74.89 aAB
			D	5242.68 aA	29.99 a	75.27 aAB
			E	5151.45 aA	30.21 a	75.58 aA
2019	BRS 374		CV (%)	13.98	11.17	2.51
			А	2369.04 cD	23.12 b	68.85 bF
			В	3682.07 bBC	29.08 a	72.68 aD
		July 11 th	C 3872.83 abBC 29.32 a		29.32 a	73.08 aCD
			D	4054.37 abBC	30.04 a	73.89 aABCD
			E	4099.18 aB	30.29 a	73.75 aBCD
			CV (%)	18.76	10.00	2.85
		CV (%) (year'	*cultivar)	21.57	10.48	2.92
			А	6070.76 nsABCD	32.86 ^{ns} A	78.27 ^{ns} A
			В	6417.63 AB	33.26 A	78.30 A
		June 16 th	С	6475.04 AB	32.70 A	77.52 ABCD
			D	6401.00 ABC	32.99 A	77.62 ABC
			E	6556.36 A	33.58 A	78.02 AB
	BRS 374		CV (%)	4.95	2.87	0.68
			А	5370.76 ^{ns} E	27.68 nsB	76.17 ^{ns} D
		June 29 th	В	5834.53 BCDE	27.78 B	77.00 ABCD
			С	5681.76 DE	28.34 B	77.20 ABCD
			D	5720.70 CDE	28.08 B	76.35 CD
			E	5868.24 ABCDE	29.83 B	76.80 BCD
			CV (%)	5.31	4.35	0.95
2020		CV (%) (year'	*cultivar)	7.67	8.57	1.15
			А	5580.52 nsAB	31.90 ^{ns} A	79.95 nsNS
			В	5732.15 A	33.39 A	80.36
		June 16 th	С	5819.49 A	32.80 A	79.96
			D	5635.89 AB	33.32 A	80.10
			Е	5529.30 AB	32.81 A	80.06
	ORS Agile		CV (%)	6.73	2.72	0.60
			A	4860.23 ^{ns} B	26.43 ^{ns} B	79.40 ^{ns}
	June 29 th		В	5400.88 AB	27.37 B	80.10
		June 29 th	С	5276.52 AB	27.41 B	79.70
			D	5212.45 AB	27.57 B	79.40
			E	5106.10 AB	28.57 B	80.20
			CV (%)	6.23	5.60	1.15
			*cultivar)			

Columns with the same lowercase letters within each sowing date, cultivar, and year show statistically similar means, according to the Tukey's HSD (α =5%). Columns with the same uppercase letters within each year and cultivar (comparison between different sowing dates) show statistically similar means, according to the Tukey's HSD (α =5%). ns/NS = not significant.

Table 4. Deoxynivalenol accumulation (DON) (ppb), severity of fusarium head blight (FHB) (%), and incidence of *Fusarium* in the kernel for the 2019 and 2020 harvest seasons, according to the sowing date, cultivar, and treatment - Chapecó, SC

Tabela 4. Acúmulo de desoxinivalenol (DON) (ppb), severidade da giberela (%) e incidência de Fusarium nos grãos para as safras 2019 e 2020 em função da época de plantio, da cultivar e tratamento - Chapecó. SC.

Year	Cultivar	Sowing date	Treatment	DON (ppb)	FHB severity (%)	Incidence of <i>Fusarium</i> i the kernel (%)
			А	1639.25 ^{ns}	0.36 ^{ns}	3.21 ^{ns}
			В	1078.75	0.10	5.21
		June 28 th	С	946.00	0.32	3.50
			D	1091.25	0.15	4.62
			E	415.25	0.10	3.93
019	BRS 374		CV (%)	61.66	200.74	85.02
			А	320.75 ^{ns}	0.98 ^{ns}	1.18 ^{ns}
			В	344.75	0.00	1.12
		July 11 th	С	406.75	0.00	1.34
			D	215.50	0.00	1.25
			E	234.25	0.00	0.53
			CV (%)	42.15	352.05	45.53
		CV (%) (yea	r*cultivar)	87.46	278.21	111.51
			А	79.90 ^{ns}	0.24 ^{ns}	0.25 ^{ns}
			В	69.20	0.01	0.43
		June 16 th	С	72.45	0.30	0.06
			D	24.50	0.01	0.09
			E	81.50	0.03	0.12
	BRS 374		CV (%)	77.07	228.96	165.44
	-	June 29 th	А	39.40 ^{ns}	0.00 ^{ns}	0.12 ^{ns}
			В	18.70	0.09	0.15
			С	36.50	0.00	0.18
			D	22.50	0.00	0.12
			E	22.50	0.00	0.00
			CV (%)	108.23	447.21	185.41
		CV (%) (yea	r*cultivar)	96.91	299.72	175.41
020			А	22.80 a	0.00 ^{ns}	0.25 ^{ns}
			В	16.30 ab	0.00	0.12
		June 16 th	С	6.30 b	0.00	0.18
			D	10.20 ab	0.00	0.00
			E	36.90 ab	0.00	0.31
	ORS Agile		CV (%)	81.80		131.91
	-		A	11.50 ^{ns}	0.00 ^{ns}	0.18 ^{ns}
		June 29 th	В	23.00	0.00	0.00
			С	12.80	0.00	0.06
			D	26.70	0.00	0.62
			E	8.70	0.00	0.00
			 CV (%)	83.91	-	318.09
		CV (%) (yea		82.00		240.35

Columns with the same lowercase letters within each sowing date, cultivar, and year show statistically similar means, according to the Mann–Whitney test (α =5%). ^{ns} = not significant.

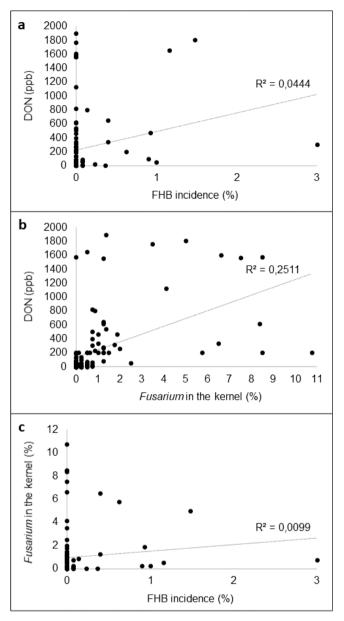


Figure 1. Pearson correlations between the variables (a) "DON accumulation × FHB severity," (b) "DON accumulation × incidence of *Fusarium* in the kernel" and (c) "incidence of *Fusarium* in the kernel × FHB severity" - Chapecó, SC

Figura 1. Correlações de Pearson entre as variáveis (a) "Acúmulo de DON × Severidade da giberela", (b) "Acúmulo de DON × Incidência de Fusarium nos grãos" e (c) "Incidência de Fusarium nos grãos × Severidade da giberela" - Chapecó, SC

contrast analysis between the combination of all control treatments (treatment A) and the combination of all other treatments (with varied fungicide applications), DON accumulation in kernels presented no significant difference (p=0.7612).

Rainfalls influenced all parameters analyzed during the study. For instance, in the 2019 harvest season, rainfall was much higher in the critical period for FHB incidence in wheat when compared with 2020 (Table 2). This influenced

yield and the quality parameters (DON accumulation, FHB severity, and incidence of *Fusarium* in the kernel) analyzed during the study.

Moreover, the harvest of wheat sown earlier in 2019 was on October 10th (thus they received less rainfall) while the harvest of wheat sown later in 2019 was on November 11th, over a month apart. On the other hand, as 2020 was a very dry year, the harvest of wheat sown both earlier and later was almost at the same time (eight days apart). This is probably why treatments performed in 2020 and 2019 presented smaller differences for the parameters analyzed.

Ducatti et al. (2022) showed that meteorologic conditions greatly affected DON accumulation in plants during the 2019, 2020, and 2021 harvest seasons in the states of Santa Catarina and Rio Grande do Sul, Brazil. We observed the same tendency during our study (Table 4). Wegulo (2012), in his review about factors influencing DON accumulation in small grain cereals, also showed the influence and importance of meteorologic conditions on FHB incidence and DON accumulation in wheat.

However, according to other studies and reviews (PONTE et al., 2012; BONFADA et al., 2019; DUCATTI et al., 2022) and this study (Figure 1), the correlation between FHB severity, incidence of *Fusarium* in the kernel, and DON accumulation is positive but weak. It shows and reinforces that DON biosynthesis and accumulation will not necessarily follow *Fusarium* contamination by the presence of *Fusarium* in wheat/kernels.

Similar amounts of *Fusarium* may biosynthesize different amounts of mycotoxins, as shown by Merhej et al. (2010), Ponte et al. (2012), Ji et al. (2015), and Bonfada et al. (2019). This is probably why the correlations between FHB severity, incidence of *Fusarium* in the kernel, and DON accumulation are small. Certainly, the best way to estimate the amount of DON accumulation in kernels, besides performing ELISA/HPLC/NIRs tests, is analyzing the presence of *Fusarium* in the kernel (a test performed in laboratory at much lower costs) (Figure 1), although the correlation between incidence of *Fusarium* in the kernel and DON accumulation is about 50%.

Thus, as mycotoxins are believed to be biosynthesized and accumulated as a fungi defense mechanism, the best way to reduce mycotoxin incidence/accumulation in kernels would be reducing fungi stress. In this sense, although fungicides are widely used to successfully control large amounts of fungi in fields, the surviving fungi will face large periods of stress, which will favor the biosynthesis of these toxins.

Moreover, inefficient fungicides and/or fungicide application during inappropriate periods or at inappropriate doses might contribute to increase DON biosynthesis and accumulation in wheat and other cereal kernels. Fungicides are essential to decrease disease pressures in fields and contribute to raise yields; however, DON biosynthesis and accumulation will not necessarily decrease because of the reduction in the amount of *Fusarium* in fields, as already mentioned.

Throughout these two years of study, treatments with fungicide application (treatments B, C, D, and E) did not significantly differ from the control treatment regarding DON accumulation in kernels. McMullen et al. (2012) collected data from more than 100 field experiments using different fungicides to control FHB and observed that different active ingredients provided different responses on the decrease in FHB, which did not largely follow the decrease in DON. Fungi are sessile organisms and, in order to defend themselves against different kinds of threats, they must produce defense metabolites. including mvcotoxins (PONTS, 2015).

Table 3 shows the achievement of greater yields mainly in the 2019 harvest season, which are linked to the use of fungicides. Although this study does not present data on other diseases that may have affected the control plots (such as powdery mildew and tan spot, for example), 2019 was a more humid year and it might have increased the incidence of other foliar diseases in the control plots. Powdery mildew, for instance, may affect wheat yield up to 40% (DRAZ et al., 2019), which is similar to the reductions observed between all treatments and the control treatment in 2019.

Conclusion

- Meteorological conditions significantly affect the incidence of diseases in wheat and influence yields and DON accumulation in kernels;

- The use of fungicides is a good disease control response and can be considered a good tool to help farmers to achieve greater yields; however, there is a low correlation between FHB severity and DON accumulation.

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