In vitro sensitivity reduction of *Fusarium graminearum* to DMI and QoI fungicides

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ABSTRACT

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In Brazil, Fusarium head blight (FHB) affecting wheat can cause up to 39.8% damage. Resistant cultivars are not available yet; thus, short-term disease control relies on the use of fungicides. The first step to improve control is to monitor fungal populations that are sensitivity to chemicals in order to achieve efficient FHB management. *In vitro* experiments were conducted to evaluate the inhibitory concentration (IC_{50}) of fungicides for both mycelial growth and conidial germination of ten *Fusarium graminearum* isolates. The following demethylation inhibitor (DMI) fungicides were tested: metconazole, prothioconazole and tebuconazole. In addition, pyraclostrobin and trifloxystrobin were included, representing QoI fungicides, as well as three co-formulations containing metconazole + pyraclostrobin, prothioconazole + trifloxystrobin, and tebuconazole + trifloxystrobin. For mycelial growth, the overall mean IC_{50} of isolates was: metconazole 0.07, prothioconazole 0.1, and tebuconazole 0.19 mg/L. For the co-formulations, it was: prothioconazole + trifloxystrobin 0.08, tebuconazole + trifloxystrobin 0.12, and metconazole + pyraclostrobin 0.14 mg/L. Regarding spore germination inhibition, IC_{50} for prothioconazole + trifloxystrobin was 0.06, for tebuconazole + trifloxystrobin, 0.12 mg/L, for QoI alone pyraclostrobin, was 0.09, and for trifloxystrobin, 0.28 mg/L. There was a sensitivity shift among isolates and the highest fungitoxicity to *F. graminearum* was confirmed for prothioconazole, metconazole and tebuconazole .

Additional keywords: Fusarium head blight, fungitoxicity, IC_{50}, *Triticum aestivum,* wheat.

RESUMO

Avozani, A.; Reis, E. M.; Tonin R. B. Redução *in vivo* da sensibilidade de *Fusarium graminearum* a fungicidas triazóis e estrobilurinas. ***Summa Phytopathologica,*** v.40, n.4, p.358-364, 2014.

Os danos causados pela giberela em trigo, no Brasil, podem chegara a 39,8%. Ainda não estão disponíveis cultivares resistentes para o controle da doença; desse modo, à curto prazo, o controle da doenças é baseado no uso de fungicidas. O primeiro passo para melhorar a eficácia do controle é monitorar a sensibilidade do fungo aos fungicidas para obter o manejo mais eficiente da doença. Experimentos foram conduzidos *in vivo* para avaliar a concentração inibitória (CI_{50}) de fungicidas tanto para o crescimento miceliano como para a germinação de conídios de dez isolados de *Fusarium graminearum*. Os seguintes fungicidas inibidores da desmetilação (IDM) foram testados: metconazol, protioconazol e tebuconazol. Também, foram testados os fungicidas inibidores da quinona externa (IQe) estrobilurinas, piraclostrobina e trifloxistrobina, bem como suas misturas contendo metconazol + piraclostrobina, protioconazol + trifloxistrobina, e tebuconazol + trifloxistrobina. A média geral da CI_{50} para o crescimento miceliano dos isolates foi: metconazol 0,07, protioconazol 0,1, e tebuconazol 0,19 mg/L. Para as misturas foi: protioconazol + trifloxistrobina 0,08, tebuconazol + trifloxistrobina 0,12, e metconazol + piraclostrobin 0,14 mg/L. Em relação a inibição da germinação dos esporos, a CI_{50} para protioconazol + trifloxistrobina foi 0,06, para o tebuconazol + trifloxistrobina, 0,12 mg/L, e para os IQes isolados piraclostrobina, 0,09, e para a for trifloxistrobina, 0,28 mg/L. Demonstrou-se haver um diferença na sensibilidade entre os isolados e confirmou-se a elevada fungitoxicidade do protioconazol, metconazol e tebuconazol à *F. graminearum*.

Palavras-chave adicionais: Giberela, fungitoxicidade, CI_{50}, *Triticum aestivum,* trigo.

Wheat (*Triticum aestivum* L.) is an important food crop in Brazil, where the annual consumption is currently around 11 million tons, of which 6 million tons are produced and 5.6 million tons are imported (5). Wheat production under prevailing warm and wet weather conditions, as in Southern Brazil, is a difficult task (15).

Fungal diseases cause considerable damage to wheat production, particularly those difficult to control, such as fusarium head blight (FHB), caused by *Gibberella zeae* (Schw.) Petch. (Anamorph *Fusarium graminearum* Schw.). FHB was first described in the United States, in 1891 (19), and in Brazil, in Veranópolis County, Rio Grande do Sul, in 1942, by Costa Netto (7).

Casa & Kuhnem Junior (4) and Reis et al. (22) reviewed the quantitative damage caused by FHB to wheat in Brazil from 1984 to 2010 growing seasons. The mean damage during this period was 18.6% and the maximum, 39.8%.

The Brazilian Health Surveillance Agency [Agência Nacional de Vigilância Sanitária] (ANVISA) (3) recently issued "Resolution-RDC No. 7", from February 18th, 2011, establishing a maximum permissible level for mycotoxin contamination in wheat food (2). Thus, the pressure for rapid development of efficient FHB control measures has been increasing.

Even though FHB has been known for a long time, there is

no control measure that reduces the quantitative and qualitative damages to sub-economic levels in Brazil and worldwide. The resistance among Brazilian cultivars is not enough to reduce the disease to a sub-economic level (21). Hence, the greatest challenge for research is to develop other immediate solutions for FHB management.

At the present time and in the short term, chemical control is the strategy of greatest potential for success; although its efficacy is still low (40-50%), it can be improved. Studies have been conducted in Brazil focusing on the chemical control of FHB, especially under field conditions, considering timing of fungicide application, rates and number of applications (4, 8, 9, 25). Thus, the sensitivity of *F. graminearum* (*Fg*) isolates to demethylation inhibitors (DMI), quinone outside inhibitors (QoI), and their co-formulations, should be annually monitored (8).

To obtain qualitative and quantitative economic control of FHB, it is fundamental to identify the most fungitoxic chemicals to be used in field applications. This task can be accomplished by *in vitro* assays as reported by Spolti et al. (26) and Avozani et al. (2).

The aim of this study was to monitor the actual sensitivity of *F. graminearum* isolates based on mycelial and spore germination sensitivity to DMI and QoI fungicides, alone or in co-formulations, which was measured by the inhibitory concentration (IC_{50}).

To evaluate *Fg* mycelial growth and conidial germination sensitivity to fungicides, the chemicals were incorporated into PSA agar medium (peeled potatoes 200g, sucrose 20g, agar 10g, 1.0 L distilled water), similarly to the method described by Russel (25).

***Fusarium graminearum* Isolates.** Ten selected monosporic isolates (Table 1) were preserved in test tubes with PSA medium, at 5°C in a refrigerator, and used throughout this study.

Tested fungicides. The following fungicides were tested: (i) metconazole (Caramba), (ii) prothioconazole (Proline), (iii) tebuconazole (Folicur 20%), the mixtures (iv) metconazole + pyraclostrobin (Opera Ultra), (v) prothioconazole + trifloxystrobin (Fox), and (vi) tebuconazole + trifloxystrobin (Nativo). For spore germination (i), prothioconazole + trifloxystrobin (Fox), tebuconazole + trifloxystrobin (Nativo), trifloxystrobin (Twist) and pyraclostrobin (Comet) were employed. These fungicides were selected in a previous study.

Mycelial growth inhibition. Seven-day-old colonies of each strain were grown on PSA, supplemented with the fungicides, after sterilization in autoclave. Five active ingredient concentrations of each fungicide were tested: 0.0, 0.001, 0.1, 1.0 and 10.0 mg/L. For the mixtures, the concentration represents the sum of both active ingredients.

On the day after the medium preparation, mycelial discs (6 mm diameter) of each isolate were placed upside down on the center of each Petri dish. The plates were sealed with PVC film and incubated in a growth chamber at 25 ± 2ºC and 12 h photoperiod for seven days.

Mycelial growth assessment. Colony growth in two perpendicular plate diameters was measured with a digital caliper when the fungal growth in the control treatment reached the plate edge. Means of the two diameters were used and converted into percent growth by comparison with the fungal growth at 0.0 mg/L (control treatment). Logarithmical regression analysis using the statistical program Costat was performed. The inhibitory concentration (IC_{50}) capable of inhibiting 50% of *Fg* mycelial growth for each isolate and fungicide was calculated from the generated equation.

Experimental design was in completely randomized factorial (fungicides x isolates), with four replicates, each experimental unit consisting of a Petri dish. The experiments were repeated twice to ensure accuracy.

Inhibition of spore germination. The surface of seven-day-old colonies grown in Petri dishes was scraped with a camel's hair brush number 20 and added of 10 mL sterile distilled water for conidium removal. Five active ingredient concentrations of the fungicide were used: 0.0, 0.001, 0.1, 1.0 and 10.0 mg/L.

On the day after the medium preparation, 350 L of a conidial suspension (10^3) was added to each Petri dish. The incubation was performed in a growth chamber at 25°C and 8 h under continuous light. The germination was stopped by adding few drops of an acetone solution containing aniline, which also stained the spores.

Evaluation of spore germination. The percentage of germination was calculated on the basis of 100 conidia per Petri dish analyzed under an optical microscope, 400 x. Conidia were considered germinated if their germ tube length was equal to or greater than the smallest spore diameter (28).

Experimental design was completely randomized with four replicates. Germination data were expressed as percentage of germination; they were subjected to logarithmic regression analysis, using the statistical program Costat. The inhibitory concentration capable of inhibiting 50% (IC_{50}) of spore germination for each isolate and fungicide was calculated by the generated equation.

Mycelial growth inhibition by DMI. Considering metconazole, IC_{50} ranged from 0.01 mg/L (for isolates 04/10, 05/10 and 07/11) to 0.15 mg/L (for isolate 06/11). Thus, isolate 06/11 required a 15-fold concentration, compared to the other three. Regarding tebuconazole, IC_{50} for isolate 02/11 was 0.03 mg/L and for isolate 01/11, 0.39 mg/L. Thus, the IC_{50} for the latter was 13 times higher than that for the former isolate. The IC_{50} for prothioconazole ranged from 0.002 (isolate 06/11) to 0.23 (isolate 09/11) with a 115-fold shift. In general, the mean IC_{50} was 0.1 mg/L for prothioconazole, 0.07 for metconazole, and 0.19 mg/L for tebuconazole. Prothioconazole was 2.7 times more potent than tebuconazole. The overall mean ranged from 0.04 mg/L (isolates 02/11 and 07/11) to 0.21 (isolate 01/11), showing a 5.25-fold sensitivity shift. The coefficient of determination R^2 ranged from 0.72 to 0.99 (Table 2).

Mycelial growth inhibition by DMI and QoI mixtures. Considering metconazole + pyraclostrobin, fungitoxicity ranged from 0.04 (isolate 07/11) to 0.33 mg/L (isolate 06/11). There was a 6.6-fold shift. As regards tebuconazole + trifloxystrobin, IC_{50} was 0.02 mg/L for isolate 02/11 and 0.26 mg/L for isolate 06/11, and

Table 1. Identification of *Fusarium graminearum* monosporic isolates from wheat kernels

Isolate/code	City - State
01/11	Castro – PR
02/11	Tapera –RS
03/11	Coxilha – RS
04/10	Londrina – PR
05/10	Palotina – PR
06/11	Passo Fundo – RS
07/11	Passo Fundo- RS
08/11	Ponta Grossa - PR
09/11	Passo Fundo –RS
10/10	Cascavel - PR

Table 2. Fungitoxicity[z] of DMI fungicides to mycelial growth of *Fusarium graminearum* isolates

Isolate	Equation, coefficient of determination and calculated IC_{50} (mg/L)[z]			Mean (CI_{50})
	Metconazole	Prothioconazole	Tebuconazole	
01/11	y = -10.8Ln(x) + 27.12	y = -10.1Ln(x) + 29.62	y = -7.06Ln(x) + 43.38	
	R^2 = 0.98	R^2 = 0.97	R^2 = 0.93	**0.21**
	IC_{50} = 0.12	**IC_{50} = 0.13**	**IC_{50} = 0.39**	
02/11	y = - 10.8Ln(x) + 17.83	y = -7.94Ln(x) + 22.16	y = - 8.41Ln(x) + 21.01	
	R^2 = 0.98	R^2 = 0.97	R^2 = 0.99	**0.04**
	IC_{50} = 0.05	**IC_{50} = 0.03**	**IC_{50} = 0.03**	
03/11	y = - 8.85Ln(x) + 30.66	y = -5.29Ln(x) + 41.35	y = -9.88Ln(x) + 35.78	
	R^2 = 0.92	R^2 = 0.72	R^2 = 0.90	**0.13**
	IC_{50} = 0.11	**IC_{50} = 0.19**	**IC_{50} = 0.24**	
04/10	y = -7.34Ln(x) + 16.92	y = -11.3Ln(x) + 28.10	y = -11.03Ln(x) + 25.32	
	R^2 = 0.96	R^2 = 0.93	R^2 = 0.99	**0.09**
	IC_{50} = 0.01	**IC_{50} = 0.14**	**IC_{50} = 0.11**	
05/10	y = - 5.96Ln(x) + 20.93	y = -11.4Ln(x) + 30.41	y = -10.99Ln(x) + 27.07	
	R^2 = 0.98	R^2 = 0.89	R^2 = 0.99	**0.10**
	IC_{50} = 0.01	**IC_{50} = 0.18**	**IC_{50} = 0.12**	
06/11	y = -10.53Ln(x) + 30.09	y = -5.08Ln(x) + 17.54	y = -8.04Ln(x) + 42.30	
	R^2 = 0.98	R^2 = 0.91	R^2 = 0.97	**0.18**
	IC_{50} = 0.15	**IC_{50} = 0.002**	**IC_{50} = 0.38**	
07/11	y = - 6.24Ln(x) + 23.36	y = -5.99Ln(x) + 25.59	y = -10.01Ln(x) + 26.39	
	R^2 = 0.89	R^2 = 0.97	R^2 = 0.97	**0.04**
	IC_{50} = 0.01	**IC_{50} = 0.02**	**IC_{50} = 0.09**	
08/11	y = -11.08Ln(x) + 24.01	y = -9.26Ln(x) + 21.88	y = -9.15Ln(x) + 40.49	
	R^2 = 0.99	R^2 = 0.99	R^2 = 0.93	**0.17**
	IC_{50} = 0.10	**IC_{50} = 0.05**	**IC_{50} = 0.35**	
09/11	y = -11.02Ln(x) + 26.78	y = -9.51Ln(x) + 36.06	y = -6.89Ln(x) + 34.95	
	R^2 = 0.99	R^2 = 0.99	R^2 = 0.91	**0.15**
	IC_{50} = 0.12	**IC_{50} = 0.23**	**IC_{50} = 0.11**	
10/10	y = -11.03Ln(x) + 19.21	y = -7.09Ln(x) + 31.99	y = - 8.95Ln(x) + 31.90	
	R^2 = 0.98	R^2 = 0.90 mg/L	R^2 = 0.99	**0.09**
	IC_{50} = 0.06	**IC_{50} = 0.08**	**IC_{50} = 0.13**	
Mean (CI_{50})	**0.07**	**0.10**	**0.19**	

[z] - Means of two experiments; y = mycelial growth; x = fungicide concentration.

the latter was 13 times higher than the former. For prothioconazole + trifloxystrobin, IC_{50} ranged from 0.003 (isolate 07/11) to 0.25 mg/L (isolate 09/11). Thus, sensitivity shift was of 83 times. The overall mean for the co-formulation metconazole + pyraclostrobin was 0.08 mg/L, while for tebuconazole + trifloxystrobin it was 0.12 mg/L or 1.75 times more potent than metconazole + trifloxystrobin (0.14 mg/L). Regarding the overall mean for the isolates, sensitivity ranged from 0.03 mg/L (isolate 07/11) to 0.2 mg/L (isolate 06/11), with a 6.67-fold shift. The coefficient of determination (R^2) varied from 0.75 to 0.99 (Table 3).

Spore germination inhibition by DMI and QoI mixtures. IC_{50} for prothioconazole + trifloxystrobin ranged from 0.03 (isolate 02/11) to 0.1 (isolate 09/11) with a 3.34-fold shift in sensitivity. For tebuconazole + trifloxystrobin, IC_{50} ranged from 0.06 (isolate 05/10) to 0.23 (isolate 02/11) mg/L, showing a 3.8-fold shift. The overall mean for prothioconazole + trifloxystrobin was 0.06 mg/L, while for tebuconazole + trifloxystrobin it was 0.12 mg/L. Thus, the mixture prothioconazole + trifloxystrobin was 2.0 times more potent than tebuconazole + trifloxystrobin. The overall mean IC_{50} for isolates ranged from 0.05 (isolates 04/10 and 07/11) to 0.13 (isolate 02/11), evidencing a 2.6-fold shift. The coefficient of determination ranged from 0.93 to 0.99 (Table 4).

Spore germination inhibition by QoI. The fungitoxicity of pyraclostrobin ranged from 0.06 (isolates 04/10 and 07/11) to 0.15 (isolate 09/11) mg/L, and isolate 09/11 was 2.5 times less sensitive. For trifloxystrobin, IC_{50} ranged from 0.08 (isolate 07/11) to 0.73 (isolate 09/11) and again isolate 09/11 was 9.13 times less sensitive.

Regarding the overall mean, pyraclostrobin (0.09 mg/L) was 3.0 times more potent than trifloxystrobin (0.28 mg/L). Pyraclostrobin was consistently more toxic for the eight isolates. The sensitivity

Table 3. Fungitoxicity of DMI and QoI mixtures to mycelial growth of *Fusarium graminearum* isolates

Isolate	Equation, coefficient of determination and calculated IC_{50} (mg/L)			Mean (CI_{50})
	Prothioconazole + trifloxystrobin	Tebuconazole + trifloxystrobin	Metconazole + pyraclostrobin	
01/11	y = - 6.52Ln(x) + 31.96	y = -9.26Ln(x) + 30.63	y = - 9.42Ln(x) + 33.23	
	R^2 = 0.93	R^2 = 0.94	R^2 = 0.92	**0.12**
	IC_{50} = 0.06	**IC_{50} = 0.12**	**IC_{50} = 0.17**	
02/11	y = - 6.55Ln(x) + 33.05	y = -5.10Ln(x) + 31.03	y = - 9.21Ln(x) + 24.51	
	R^2 = 0.85	R^2 = 0.91	R^2 = 0.99	**0.05**
	IC_{50} = 0.08	**IC_{50} = 0.02**	**IC_{50} = 0.06**	
03/11	y = - 5.80Ln(x) + 37.33	y = -6.76Ln(x) + 27.50	y = -7.80n(x) + 40.74	
	R^2 = 0.87	R^2 = 0.85	R^2 = 0.87	**0.15**
	IC_{50} = 0.11	**IC_{50} = 0.04**	**IC_{50} = 0.31**	
04/10	y = - 7.30Ln(x) + 29.01	y = -9.56Ln(x) + 32.54	y = -10.93Ln(x) + 24.45	
	R^2 = 0.89	R^2 = 0.99	R^2 = 0.99	**0.11**
	IC_{50} = 0.06	**IC_{50} = 0.16**	**IC_{50} = 0.12**	
05/10	y = - 6.98Ln(x) + 33.00	y = - 6.93Ln(x) + 33.21	y = -10.33Ln(x) + 26.39	
	R^2 = 0.88	R^2 = 0.99	R^2 = 0.98	**0.10**
	IC_{50} = 0.09	**IC_{50} = 0.09**	**IC_{50} = 0.11**	
06/11	y = -4.24Ln(x) + 31.18	y = -9.18Ln(x) + 37.62	y = -8.86Ln(x) + 40.06	
	R^2 = 0.98	R^2 = 0.99	R^2 = 0.99	**0.20**
	IC_{50} = 0.01	**IC_{50} = 0.26**	**IC_{50} = 0.33**	
07/11	y = - 4.72Ln(x) + 22.19	y = -10.17Ln(x) + 19.98	y = -9.65Ln(x) + 18.12	
	R^2 = 0.75	R^2 = 0.99	R^2 = 0.98	**0.03**
	IC_{50} = 0.003	**IC_{50} = 0.05**	**IC_{50} = 0.04**	
08/11	y = -6.54Ln(x) + 15.45	y = - 8.50Ln(x) + 37.11	y = -11.19Ln(x) + 24.10	
	R^2 = 0.99	R^2 = 0.96	R^2 = 0.99	**0.11**
	IC_{50} = 0.005	**IC_{50} = 0.22**	**IC_{50} = 0.10**	
09/11	y = -9.36Ln(x) + 36.93	y = -9.84Ln(x) + 30.07	y = -10.37Ln(x) + 23.69	
	R^2 = 0.96	R^2 = 0.98	R^2 = 0.97	**0.15**
	IC_{50} = 0.25	**IC_{50} = 0.13**	**IC_{50} = 0.08**	
10/10	y = -9.85Ln(x) + 32.92	y = -8.14Ln(x) + 33.65	y = - 10.5Ln(x) + 18.42	
	R^2 = 0.98	R^2 = 0.99	R^2 = 0.93	**0.12**
	IC_{50} = 0.18	**IC_{50} = 0.13**	**IC_{50} = 0.05**	
Mean (CI_{50})	**0.08**	**0.12**	**0.14**	

[y] - Means of two experiments; y = mycelial growth; x = fungicide concentration.

of isolates ranged from 0.07 (isolate 07/11) to 0.44 (isolate 09/11) with a 6.3-fold shift in sensitivity. The coefficient of determination (R^2) ranged from 0.94 to 0.99 (Table 5).

Until 2013, no FHB-resistant or tolerant cultivars were available. Subsequently, McMullen et al. (16) emphasized that "crop rotations are the key to reducing risk of severe scab". Nevertheless, the crop rotation effectiveness in reducing scab has not been demonstrated in Brazil (23). Likewise, in Argentina, Moschini et al. (18) suggested that favorable weather conditions are likely to be more important than tillage practice for disease severity. Thus, FHB is a disease difficult to manage and, under environmental conditions favorable to the pathogen, the use of only one management strategy may result in management failure.

The alternative is that resistance will be discarded when environmental conditions become favorable for scab. Therefore, while resistant/tolerant cultivars are not available yet, a more feasible alternative for FHB control is to improve the efficiency of chemical control.

In general, fungicides containing triazole as the active ingredient (metconazole, prothioconazole and tebuconazole) are considered the most effective chemical compounds against *Fg* (8, 12, 15, 17, 20, 21, 27).

The sensitivity of isolates to fungicides can be classified according to the standard criteria of Edgington et al. (10), considering the following attributes: insensitive when IC_{50} >50 mg/L; moderately sensitive when IC_{50} is between 1 and 10 mg/L; highly sensitive when IC_{50} <1 mg/L. According to this classification, all ten isolates could be considered sensitive for mycelial and spore germination, based on the IC_{50} of trifloxystrobin and prothioconazole (0.08 to 0.44 mg/L). Isolate 03/11 was the most sensitive to azoxystrobin with an IC_{50} of 0.03 mg/L, whereas isolate 04/10 was the least sensitive to trifloxystrobin, with an IC_{50} of 2.71 mg/L.

Metconazole, prothioconazole and tebuconazole were the

Table 4. Fungitoxicity of DMI fungicide mixtures to spore germination of *Fusarium graminearum* isolates

Isolates	Equation, coefficient of determination and calculated IC_{50} (mg/L)[y]		Mean (CI_{50})
	Prothioconazole + trifloxystrobin	Tebuconazole + trifloxystrobin	
01/11	-	-	-
02/11	y = -7.72 Ln(x) + 23.59	y = - 9.17Ln(x) + 36.72	
	R^2 = 0.96	R^2 = 0.95	**0.13**
	IC_{50} = 0.03	**IC_{50} = 0.23**	
03/11	-	-	-
04/10	y = - 9.02Ln(x) + 21.97	y = - 8.91Ln(x) + 25.97	
	R^2 = 0.96	R^2 = 0.99	**0.05**
	IC_{50} = 0.04	**IC_{50} = 0.07**	
05/10	y = -9.90Ln(x) + 22.26	y = - 9.65Ln(x) + 22.27	
	R^2 = 0.96	R^2 = 0.98	**0.06**
	IC_{50} = 0.06	IC_{50} = 0.06	
06/11	y = - 9.47Ln(x) + 21.95	y = - 7.49Ln(x) + 37.05	
	R^2 = 0.96	R^2 = 0.99	**0.11**
	IC_{50} = 0.05	**IC_{50} = 0.18**	
07/11	y = -9.25Ln(x) + 20.45	y = - 6.25Ln(x) + 33.39	
	R^2 = 0.95	R^2 = 0.93	**0.05**
	IC_{50} = 0.04	**IC_{50} = 0.07**	
08/11	Y = - 9.26Ln(x) + 21.37	y = - 8.69Ln(x) + 32.17	
	R^2 = 0.98	R^2 = 0.94	**0.09**
	IC_{50} = 0.05	**IC_{50} = 0.13**	
09/11	Y = - 9.35Ln(x) + 28.09	y = - 9.46Ln(x) + 28.28	
	R^2 = 0.99	R^2 = 0.99	**0.10**
	IC_{50} = 0.10	**IC_{50} = 0.10**	
10/10	Y = - 9.29Ln(x) + 26.64	y = - 9.21Ln(x) + 29.52	
	R^2 = 0.98	R^2 = 0.99	**0.09**
	IC_{50} = 0.08	**IC_{50} = 0.11**	
Mean (CI_{50})	**0.06**	**0.12**	

[y] - Means of two experiments; y = spore germination; x = fungicide concentration.
[z] - no sporulation.

most potent DMIs, providing more efficient mycelium growth inhibition, with IC_{50} <0.01 mg/L (2). All tested fungicides showed fungitoxicity for mycelial growth; IC_{50} was <1 mg/L for all isolates. Nevertheless, the isolates showed, as expected, different sensitivity to the fungicides. Isolates 02/11 and 07/11 were most sensitive, whereas isolate 06/11 can be considered the least sensitive isolate (Tables 2, 3, 4).

Thus, metconazole, prothioconazole, tebuconazole and their mixtures with pyraclostrobin and trifloxystrobin can be used in FHB control. However, fungicide deposition should be improved to reach and cover the head sides (4).

Spolti et al. (6) determined an IC_{50} of 0.001 mg/L (< 0.001-0.152 mg/L) and 0.037 mg/L (< 0.001-0.324) for tebuconazole and metconazole alone, respectively. When these IC_{50} values are compared with those obtained in our study, the value for tebuconazole (mean of ten isolates, IC_{50} 0.19 mg/L) was 190 times higher, and that for metconazole (IC_{50} 0.07 mg/L) was 70 times higher than the IC_{50} determined in Spoltis` study with *Fg* isolates collected in 2009 and 2010 growing seasons.

Of the fungicides most widely tested against this disease, the triazole group (e.g., tebuconazole) has been the most effective, yielding the most consistent results (20, 21. 26, 28, 30). However, even among triazoles or for a given triazole, efficacy has varied among studies (24).

As the mode of action should be the same for all tested triazoles, the large difference in effect between epoxiconazole and the other triazoles is remarkable (1, 13).

Chemical control can reduce not only FHB damage, but also wheat contamination with mycotoxins (8, 11, 12, 14, 21).

Of the fungicides tested alone, the most powerful were prothioconazole and pyraclostrobin; thus, their mixture should be the most indicated use for producers.

Therefore, it is not sufficient to have a highly fungitoxic fungicide if the spray technology is not efficient enough to completely cover the infection sites located at the head sides.

The isolates are still sensitive (10) to the tested fungicides, showing an IC_{50} lower than 1.0 mg/L, but a sensitivity shift among the ten tested isolates was shown to DMI and to QoI.

Table 5. Fungitoxicity of QoI fungicides to spore germination of *Fusarium graminearum* isolates

Isolates	Equation, coefficient of determination and calculated IC_{50} (mg/L)[y]		Mean (CI_{50})
	Pyraclostrobin	Trifloxystrobin	
01/11	-	-	**-**
02/11	y = - 8.55Ln(x) + 30.54	y = - 6.94Ln(x) + 39.38	
	R^2 = 0.99	R^2 = 0.99	**0.16**
	IC_{50} = 0.10	**IC_{50} = 0.22**	
03/11	-	-	**-**
04/10	y = - 9.73Ln(x) + 22.00	y = - 7.82Ln(x) + 32.30	
	R^2 = 0.96	R^2 = 0.98	**0.08**
	IC_{50} = 0.06	**IC_{50} = 0.10**	
05/10	y = - 8.94Ln(x) + 27.55	y = - 8.71Ln(x) + 32.76	
	R^2 = 0.96	R^2 = 0.99	**0.11**
	IC_{50} = 0.08	**IC_{50} = 0.14**	
06/11	y = - 8.45Ln(x) + 30.82	y = - 6.95Ln(x) + 42.11	
	R^2 = 0.99	R^2 = 0.98	**0.21**
	IC_{50} = 0.10	**IC_{50} = 0.32**	
07/11	y = - 9.26Ln(x) + 23.49	y = - 8.94Ln(x) + 27.49	
	R^2 = 0.96	R^2 = 0.96	**0.07**
	IC_{50} = 0.06	**IC_{50} = 0.08**	
08/11	y = - 8.31Ln(x) + 30.45	y = - 7.96Ln(x) + 40.87	
	R^2 = 0.99	R^2 = 0.97	**0.21**
	IC_{50} = 0.10	**IC_{50} = 0.32**	
09/11	y = - 8.80Ln(x) + 33.11	y = - 7.31Ln(x) + 47.74	
	R^2 = 0.99	R^2 = 0.94	**0.44**
	IC_{50} =0.15	**IC_{50} = 0.73**	
10/10	y = - 8.57Ln(x) + 30.69	y = - 7.77Ln(x) + 41.45	
	R^2 = 0.99	R^2 = 0.98	**0.22**
	IC_{50} = 0.11	**IC_{50} = 0.33**	
Mean (CI_{50})	**0.09**	**0.28**	

[y] - Means of two experiments; y = spore germination; x = fungicide concentration.
[z] - no sporulation.

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