Reduction in the *in vitro* sensitivity oF *Drechslera tritici-repentis*, isolated from wheat, to strobilurin and triazole fungicides

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ABSTRACT

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Reports of failure in the chemical control of wheat yellow leaf spot led to determination of the sensitivity of *Drechslera tritici-repentis* (*Dtr*) to the fungicides quinone outside inhibitors (QoIs) and demethylation inhibitors (DMIs). The IC_{50} was obtained for strobilurins (azoxystrobin, kresoxim-methyl, picoxystrobin and pyraclostrobin) and for triazoles (cyproconazole, epoxiconazole, propiconazole, prothioconazole and tebuconazole), using five *Dtr* isolates. Seven concentrations of the fungicides were tested in the bioassay: 0.00; 0.01; 0.10; 1.00; 10:00 and 20.00 and 40.00 mg/L active ingredient (a.i.). Assays consisted of completely randomized design and four replicates. Each experiment was performed twice, using the average of the two tests for statistical analysis. The percentage inhibition data for conidial germination (QoIs) and for mycelial growth (DMIs) were subjected to logarithmic regression analysis, calculating the 50% inhibitory concentration (IC_{50}) based on the generated equation. There was a reduction in the sensitivity of *Dtr* isolates to strobilurins. IC_{50} values ranged from 0.58 to > 40.00 mg/L. The lowest sensitivity of isolates was detected for azoxystrobin, kresoxim-methyl, picoxystrobin and trifloxystrobin. Pyraclostrobin was most efficient, showing IC_{50} between 0.58 and 1.03 mg/L. The IC_{50} ranged from 0.35 to 1.37 mg/L for epoxiconazole, from 0.49 to 1.28 mg/L for propiconazole and from 1.41 to 2.34 mg/L for tebuconazole. Prothioconazole was most potent, showing IC_{50} between 0.09 and 0.21 mg/L. The hypothesis that the control failure can be attributed to the reduced *Dtr* sensitivity to the fungicides QoIs and DMIs was confirmed.

Keywords: IC_{50}, fungitoxicity, QoIs, yellow spot*, Pyrenophora tritici-repentis, Triticum aestivum*.

RESUMO

Tonin, R. B; Reis, E. M.; Avozani, A. Redução da sensibilidade *in vitro* de *Drechslera tritici-repentis*, isolados do trigo, a fungicidas estrobilurinas e triazóis, *in vitro*. ***Summa Phytopathologica,*** v.43, n.1, p.20-25, 2017.

Devido a relatos da ocorrência de falha de controle químico da mancha-amarela da folha do trigo, determinou-se a sensibilidade de *Drechslera tritici-repentis* (*Dtr*) aos fungicidas inibidores da quinona externa (IQe) e aos inibidores da desmetilação (IDMs). A CI_{50} foi determinada para as estrobilurinas (azoxistrobina, cresoxim-metílico, picoxistrobina e piraclostrobina), para os triazóis (ciproconazol, epoxiconazol, propiconazol, protioconazol e tebuconazol) e usando cinco isolados de *Dtr* de trigo. Sete concentrações dos fungicidas foram testadas no bioensaio: 0,00; 0,01; 0,10; 1,00; 10,00; 20,00 e 40,00 mg/L de ingrediente ativo (i.a.). Os ensaios constituiuram-se de delineamento inteiramente casualizados, com quatro repetições. Cada experimento foi realizado duas vezes utilizando-se para a análise estatística as médias dos dois testes. Os dados da porcentagem da inibição da germinação de conídios (IQes) e do crescimentoe do micélio (IDMs) foram submetidos à análise de regressão logarítmica, calculando-se a concentração inibitória de 50 % (CI_{50}) através da equação gerada. Foi verificada redução da sensibilidade de isolados de *Dtr* para as estrobilurinas. Valores da CI_{50} situaram-se entre 0,58 a > 40,00 mg/L. A menor sensibilidade dos isolados, foi detectada para azoxistrobina, cresoxim-metílico, picoxistrobina e trifloxistrobina. A piraclostrobina mostrou-se o mais eficiente, com CI_{50} entre 0,58 a 1,03 mg/L. A CI_{50} para o epoxiconazol situou-se entre 0,35 a 1,37 mg/L, para propiconazol de 0,49 a 1,28 mg/L e de 1,41 a 2,34 mg/L para tebuconazol. O protioconazol foi o mais potente, com CI_{50} entre 0,09 a 0,21 mg/L. Confirmou-se a hipótese de que a falha de controle pode ser atribuída a redução da sensibilidade de *Dtr* aos fungicidas IQes e IDMs.

Palavras-chave: $CI_{50,}$ fungitoxicidade, IQes*,* mancha-amarela*, Pyrenophora tritici-repentis, Triticum aestivum*

The fungus *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera ritici-repentis* (Died.) Shoem. is the causal agent of yellow leaf spot (YLS) of wheat (12, 15, 16). This disease has been a threat to wheat production in many countries (12, 14, 15, 16). Damage caused to wheat by YLS can be calculated based on the normalized function Y = 1,000 - 5.7 I (Y = yield kg/ha and I = leaf incidence) (16).

In Southern Brazil, YLS has been predominant among wheat diseases in recent seasons (27). It is related to no-tillage and wheat monoculture since this pathogen is a necrotrophic fungus that survives in crop debris in its teleomorph state and in other hosts such as rye (*Secale cereale* L.) and triticale (*Triticum secalotricum* Meister) (14).

Integrated management of YLS is achieved by the use of tolerant cultivars, healthy seeds, seed lots treated with efficient fungicide, crop rotation and fungicide application on the foliage (16). Genetic resistance is a very important control measure; however, in Brazil, a resistant cultivar to control YLS of wheat is not available (23).

Two fungicide groups have been used to control wheat diseases, triazoles and strobilurins. Among triazoles or demethylation inhibitors

(DMI), cyproconazole, epoxiconazole and tebuconazole have been intensively employed by farmers to control the pathogen; their use alone or in mixture with quinone outside inhibitors (QoI) or strobilurins is recommended (3, 7, 9). The fungicide propiconazole was first used in the 1986 growing season (18), tebuconazole in the 1991 (19, 20), cyproconazole in the 1993 (21), and epoxiconazole in the 2000 growing season (22). After fifteen years of triazole use, the first complaint of failure to control leaf rust caused by *Puccinia triticina* Eriks. was reported (2).

Fungicide application is one of the main methods to control plant diseases; however, repeated use in different growing seasons can promote the selection of resistant strains of pathogenic fungi (11). Currently, sensitivity reduction has been one of the most important problems faced by the chemical control of plant diseases (4, 6, 11, 25).

The sensitivity of a fungus to a certain fungicide or the chemical fungitoxicity is measured by adopting parameters such as ED_{50} (effective dose to promote a desired effect in 50% of the microorganisms subjected to the test), LD_{50} (lethal dose), LC_{50} (lethal concentration), EC_{50} (effective concentration) MIC (minimum inhibitory concentration), GI_{50} (growth inhibition) and IC_{50} (inhibitory concentration to inhibit by 50% the mycelial growth and/or spore germination) (11, 25).

There are few studies monitoring the sensitivity of *Drechslera ritici-repentis (Dtr)* to fungicides recommended by the Brazilian wheat research to pathogen control. Fungicides have been intensively employed by farmers, and their mixtures DMI + QoI are recommended for pathogen control (23).

The shift in sensitivity of a pathogen to a specific fungicide can compromise the efficacy of chemical control of diseases such as leaf rust (2) and powdery mildews (17). Thus, monitoring the sensitivity of a pathogen population to fungicides is important to maintain the control efficiency.

A large number of farm advisers and growers have complained about failure in the chemical control of YLS in the last growing seasons after continuous use of fungicides for almost 20 years. The first step towards a scientific explanation for control failure is to measure the sensitivity of the fungus to the commonly used fungicide.

We hypothesized that YLS control failure could be due to the shift in the pathogen sensitivity to fungicides used in the wheat field for a long time.

The aim of this study was to determine the sensitivity of *Dtr* isolates from wheat to QoI and DMI fungicides considering spore germination and mycelial growth.

MATERIAL AND METHODS

Isolates of *Drechslera tritici-repentis.* The isolates were obtained from wheat leaves showing symptoms of the disease, collected from fields in the states of Paraná and Rio Grande do Sul (Table 1).

Table 1. Origin and identification of *Drechslera tritici-repentis* isolates

Isolate	Wheat cultivar	County/State	Identification
01	Quartzo	Ventania – PR	01/QTZ
02	Onix	Santo Augusto – RS	02/ONX
03	Horizonte	Júlio de Castilhos – RS	03/HZT
04	Guamirim	Coxilha – RS	04/GUA
05	CD 104	Pitangueiras – PR	05/CD

Test with strobilurins - Spore germination. To evaluate the sensitivity of *Dtr* spore germination to fungicides, a bioassay was performed by incorporating the fungicides in water agar medium, similarly to the method described by Russell (2004).

Fungicides. The following QoI fungicides were used in the test: azoxystrobin (Priori 250 SC), kresoxim-methyl (Stroby 500 SC), picoxystrobin (Oranis 250 SC), pyraclostrobin (Comet 250 EC) and trifloxystrobin (Twist 125 CE). These fungicides are recommended to be used in mixture (QoIs + DMIs) for the control of YLS in wheat, except picoxystrobin (Reunião, 2011).

Fungicides were initially diluted in sterile distilled water (SDW) to obtain the desired concentrations. Six active ingredient concentrations were used in the bioassay: 0.00, 0.01, 0.10, 1.00, 10.00, 20.00 and 40.00 mg/L. The 0.00 mg/L concentration, or no fungicide, represented the control of each experiment.

Spore suspensions of each *Dtr* isolate were prepared by using colonies with abundant sporulation of the pathogen after seven days of growth in V8 agar medium. Spores were removed by scraping the colony with a camel's hair brush number 20, containing approximately 10 ml of sterile distilled water. A 350-µL aliquot of the spore suspension of each isolate was poured onto each Petri dish containing the medium supplemented with the fungicide concentrations. Petri dishes were kept in a BOD incubator (Biological Oxygen Demand) (Marconi, Piracicaba, SP) at 22 ± 1 °C, under continuous light provided by three fluorescent lamps OSRAM Universal, 40 W, at 15 cm above the plates for eight hours.

Spore germination assessment. After eight-hour exposure, germination was stopped by adding drops of acetone (100%) plus a cotton blue dye in each Petri dish. Germination assessment was performed by scanning at random 100 conidia per plate under an optical microscope, 400X magnification, per replicate. Conidia showing a germ tube length equal to or greater than the smallest spore diameter were considered germinated (28).

Test with DMI fungicides – Mycelial inhibition. The DMI fungicides cyproconazole (Alto 100, 100 SL), epoxiconazole (Opus 125 SC), propiconazole (Tilt 250 EC), prothioconazole (Proline 250 EC) and tebuconazole (Folicur 200 EC) were used in the test. These fungicides are recommended to be used in mixture with QoIs to control YLS in wheat crop (20). Prothioconazole, a novel fungicide, was employed to compare its performance with that of the traditionally used fungicides.

The concentrations of each assessed fungicide were: 0.00; 0.01; 0.10; 1.00; 10.00; 20.00 and 40.00 mg/L a.i. For dilution, fungicide aliquots were transferred with a micropipette to a flask containing sterile distilled water (SDW), resulting in a final volume of 100 ml (base 1 suspension). One mL from this first suspension was transferred to 99.0 mL SDW in a volumetric flask, constituting the second dilution solution (base 2 suspension). Required volumes of the base 2 suspension were added to wheat leaf extract agar medium (WLEA) (3 g wheat leaf, 20 g dehydrated PDA, 8 g agar, 1.0 L distilled water) to obtain the desired concentrations. Flasks were carefully shaken and the supplemented medium was poured onto sterilized Petri dishes (90 diameter x 15 mm height) in a laminar flow hood.

Discs of 5 mm diameter containing fungal mycelium, taken from the edge of the seven-day-old colonies, were transferred to Petri dishes containing WLEA supplemented with the fungicide concentrations. Plates were incubated in a growth chamber at 25 ± 2°C, 12 h photoperiod; light was provided by three fluorescent lamps, 40 W, at 50 cm above the plates, which were randomly distributed on shelves.

Mycelial growth assessment. Mycelial inhibition was obtained by measuring with a digital caliper (Mitutoyo) the radial growth of

colonies in two perpendicular diameters when the fungal growth in the control treatment reached the plate edge.

Experimental design. A complete randomized factorial design (5 fungicides x 5 isolates) was adopted with four replicates, and each experimental unit was represented by a Petri dish. Experiments were repeated twice and means were used in statistical analysis. Mycelial growth data, as centimeters, were converted into percent inhibition.

Data analysis. Data were subjected to logarithmic regression analysis and the concentration that inhibits 50% spore germination or mycelium growth (IC_{50}) was calculated, using the statistical program Costat.

Classification of isolates' sensitivity. Isolates were classified according to Edgington et al. (8) adapted to the following criteria: insensitive, IC_{50} > 40 mg/L; low sensitivity, IC_{50} between 10 and 40 mg/L; moderately sensitive, IC_{50} between 1 and 10 mg/L; highly sensitive, IC_{50} < 1 mg/L. The IC_{50} is defined as the concentration of active ingredient that inhibits by 50% spore germination or mycelial growth.

Sensitivity reduction factor. To detect the magnitude of the fungus shift in sensitivity to fungicides, the sensitivity reduction factor (SRF) was calculated by dividing the IC_{50} of the suspected isolate by the IC_{50} of the most sensitive isolate. When the SRF value was equal to 1, there was no shift in sensitivity, and when the SRF value was > 1, there was sensitivity reduction

RESULTS

***Drechslera tritici-repentis* sensitivity to strobilurins.** The IC_{50} values for 01/QTZ isolate ranged from 0.75 to > 40 mg/L. Based on the average of the two experiments, this isolate was considered sensitive to pyraclostrobin (0.75 mg/L) , showing IC_{50} lower than 1.0 mg/L, and was insensitive to azoxystrobin, kresoxim-methyl, trifloxystrobin and picoxystrobin, showing IC_{50} > 40 mg/L (Table 2).

02/ONX isolate was considered insensitive, showing values greater than 1 mg/L for azoxystrobin, kresoxim-methyl, trifloxystrobin and picoxystrobin (> 40 mg/L). Values lower than 1.0 mg/L were found for pyraclostrobin (0.85 mg/L). This isolate was classified as sensitive to this active ingredient (Table 2).

03/HTZ isolate was considered sensitive to pyraclostrobin and insensitive to the other fungicides (Table 2).

04/GUA isolate was highly sensitive to pyraclostrobin (0.58 mg/L). Values greater than 1.0 mg/L were found for azoxystrobin, kresoxim-methyl, trifloxystrobin and picoxystrobin (> 40 mg/L). Thus, this isolate was considered insensitive to these active ingredients (Table 2).

The IC_{50} for 05/CD isolate ranged from 1.03 to > 40 mg/L. This isolate was classified as insensitive to azoxystrobin (> 40 mg/L), kresoxim-methyl (> 40 mg/L), picoxystrobin (> 40 mg/L), pyraclostrobin (1.03 mg/L) and trifloxystrobin (> 40 mg/L), which means that these active ingredients were nontoxic to this isolate, demonstrating a sensitivity reduction to these fungicides (Table 2).

Interaction between isolates and fungicides was significant (p <0.05) (Table 4). On average, pyraclostrobin showed the lowest IC_{50} for the five isolates, ranging from 0.58 to 1.03 mg/L. On the other hand, the IC_{50} for azoxystrobin, kresoxim-methyl, trifloxystrobin and pycoxistrobin was higher than 40 mg/L (Table 2).

The lowest IC_{50} was determined for 04/GUA isolate when tested with pyraclostrobin, the most fungitoxic fungicide, showing IC_{50} of 0.58 mg/L (Table 2).

None of the active ingredients, azoxystrobin, kresoxim-methyl, picoxystrobin and trifloxystrobin, inhibited 100% spore germination using the six tested concentrations. Moreover, the fungicide pyraclostrobin showed crescent inhibition as the active ingredient concentration increased.

***Drechslera tritici-repentis* sensitivity to DMIs.** Regarding the effects of cyproconazole, epoxiconazole, propiconazole, prothioconazole and tebuconazole, none of them inhibited 100% fungal mycelial growth at the concentrations of 0.01, 0.1 and 1.0 mg/L (Table 3).

The sensitivity of fungal isolates was classified based on the standard criterion of Edgington et al. (8). After analyzing our data, a modified classification was proposed: IC_{50} > 40 mg/L, insensitive; IC_{50} between 10 and 40 mg/L, low sensitivity; IC_{50} between 1 and 10 mg/L, moderately sensitive, and IC_{50} < 1 mg/L, highly sensitive isolate (Table 3).

01/QTZ isolate was considered sensitive according to our classification, showing IC_{50} values lower than 1.0 mg/L for prothioconazole and propiconazole. IC_{50} ranged from 0.86 mg/L for propiconazole and 0.18 mg/L for prothioconazole. The strain was considered moderately sensitive to the fungicides epoxiconazole, IC_{50} value of 1.37 mg/L, and tebuconazole, IC_{50} value of 1.84 mg/L. In addition, the strain was considered insensitive to the active ingredient cyproconazole, showing IC_{50} value > 40 mg/L (Table 3).

02/ONX isolate was highly sensitive to epoxiconazole and prothioconazole. Its IC_{50} values ranged from 0.35 mg/L for epoxiconazole and 0.16 mg/L for prothioconazole. Values greater than 1.0 mg/L were observed for propiconazole, between 1.28 mg/L and 2.34 mg, and for tebuconazole; the isolate was considered moderately sensitive to these active ingredients and classified to have low sensitivity to cyproconazole (IC_{50} of 28.11 mg/L) (Table 3).

Table 2. Concentrations (mg/L) of QoI fungicides to inhibit 50% spore germination (IC_{50}) of *Drechslera tritici-repentis* isolates

Fungicide	Isolates (CI_{50} mg/L)					Mean
	01/QTZ	02/ONX	03/HZT	04/GUA	05/CD	
Azoxystrobin	A > 40 a	A > 40 a	A > 40 a	A > 40 a	A > 40 a	> 40 a
Kresoxim methyl	A > 40 a	A > 40 a	A > 40 a	A > 40 a	A > 40 a	> 40 a
Picoxystrobin	A > 40 a	A > 40 a	A > 40 a	A > 40 a	A > 40 a	> 40 a
Pyraclostrobin	D 0.75 b	B 0.85 b	C 0.78 b	E 0.58 b	A 1.03 b	0.80 b
Trifloxystrobin	A > 40 a	A > 40 a	A > 40 a	A > 40 a	A > 40 a	> 40 a
Mean	>32.15	32.17	>32.15	>32.11	>32.20	
CV (%)	0.02					

Means followed by the same letter do not differ according to Tukey's test at 5%. Lowercase letters compare means in the column and capital letters, in the lines. Means of two experiments,

Table 3. Concentrations (mg/L) of DMI fungicides to inhibit 50% mycelial growth (IC_{50}) of *Drechslera tritici-repentis* isolates

Fungicide	Isolates					Mean
	01/QTZ	02/ONX	03/HZT	04/GUA	05/CD	
Cyproconazole	A > 40.0 a	C 28.11a	A > 40.0 a	A > 40.0 a	B 34.08 a	> 36.44a
Epoxiconazole	A 1.37 bc	C 0.35 d	AB 1.11 c	BC 0.63 c	C 0.45 d	0.78 c
Propiconazole	AB 0.86 c	A 1.28 c	B 0.49 cd	B 0.50 c	A 1.17 c	0.86 c
Prothioconazole	A 0.18 d	A 0.16 d	A 0.21 d	A 0.09 c	A 0.17 d	0.16 d
Tebuconazole	AB 1.84 b	A 2.34 b	AB 1.82 b	B 1.41 b	A 2.21b	1.94 b
Mean	> 8.85 a	6.45 d	> 8.72 ab	> 8.52 b	7.42 c	
CV (%)	2.77					

Means followed by same letter do not differ according to Tukey's test at 5%. Capital letters compare means in the column and lowercase letters, in the line. Mean of two experiments.

03/HTZ isolate was classified as insensitive to cyproconazole, showing IC_{50} value > 40 mg/L, the lowest sensitivity to this fungicide. Similar results were found for the other strains. IC_{50} values ranged from 0.49 mg/L for propiconazole to 0.21 mg/L for prothioconazole. This strain was considered moderately sensitive to the fungicides epoxiconazole (IC_{50} = 1.11 mg/L) and tebuconazole (IC_{50} =1.82 mg/L) (Table 3).

04/GUA isolate had IC_{50} < 1 mg/L for 0.63 mg/L epoxiconazole, 0.50 mg/L propiconazole and 0.09 mg/L prothioconazole. The isolate was considered sensitive to these fungicides. At 10 mg/L, mycelial growth inhibition was 100% for prothioconazole. The strain was considered moderately sensitive to tebuconazole, showing IC_{50} of 1.41 mg/L, and insensitive to cyproconazole, showing IC_{50} of 40 mg/L (Table 3).

For 05/CD strain, IC_{50} ranged from 0.17 to 34.08 mg/L for the five fungicides. The strain was classified as sensitive, showing values lower than 1mg/L for epoxiconazole (0.45 mg/L) and prothioconazole (0.17 mg/L). Values greater than 1.0 mg/L were determined for propiconazole, tebuconazole and cyproconazole. The IC_{50} values were 1.17 mg/L (propiconazole) and 2.21 mg/L (tebuconazole), and the isolate was considered moderately sensitive to these active ingredients. Low sensitivity was detected for cyproconazole, IC_{50} of 34.08 mg/L (Table 3).

Interaction between isolates and fungicides was significant (p <0.05) (Table 3). Among the five DMI fungicides tested *in vitro*, prothioconazole showed the lowest IC_{50} values for the five isolates; thus, it was the most efficient fungicide in inhibiting mycelium growth of *Dtr*. The obtained values varied from 0.09 to 0.21 mg/L (Table 3). At 10 mg/L, mycelial growth of the five isolates was completely inhibited.

The lowest IC_{50} was observed for the strain 04/GUA to prothioconazole (0.09 mg/L). This fungicide was the most fungitoxic, presenting IC_{50} value of 0.09 mg/L (not statistically different from that of epoxiconazole and propiconazole). In the literature, no report on *in vitro* fungitoxicity and IC_{50} values was found for prothioconazole to *Dtr* isolated from wheat; however, this chemical can be highly promising in controlling the disease, according to our results (Table 3).

The highest IC_{50} values were determined for cyproconazole, between 28.11 mg/L and > 40.0, indicating that, *in vitro*, the isolates were less sensitive to this fungicide. There was fungal mycelium growth, expressed as colony diameter, for all tested concentrations and for the five isolates (Table 3).

The active ingredient tebuconazole was classified as moderately fungitoxic to all tested isolates, statistically different from the other fungicides, except for 01/QTZ isolate to epoxiconazole (Table 3).

In the overall mean, prothioconazole was the most potent fungicide, followed by epoxiconazole, propiconazole and tebuconazole (Table 3).

Comparing the sensitivity of isolates, in the overall mean, to the five fungicides, 01/QTZ isolate from Ventania, PR, was the least sensitive but did not differ statistically from 03/HTZ from Julio de Castilhos, RS. 02/ONX from St. Augusto, RS, was the most sensitive isolate (Table 3).

SRF is a useful tool to quantify the shift in sensitivity of a fungus to a fungicide (11). Considering SRF, there was a reduction in sensitivity for the studied isolates. On average, among isolates, 01/QTZ from Ventania, PR, showed the highest SRF (2.24), and 04/GUA from Coxilha, RS, had the lowest SRF (1.20) (Table 4).

In the overall mean, SRF ranged from 1.36 to 2.23, i.e., the isolates require a fungicide concentration from 1.36 to 2.23 times higher than that of the most sensitive isolate to obtain 50% reduction in the fungal mycelial growth (Table 3).

The development of fungal sensitivity shift to DMI is gradual and slow due to the polygenic or quantitative resistance type (4, 5, 6, 7, 10). According to the same authors, fungal sensitivity reduction to DMI fungicides can be reversed to a condition of a higher fungal sensitivity when these fungicides are less extensively used and/or when alternative fungicides are used to control the disease.

DISCUSSION

***Drechslera tritici-repentis* sensitivity to triazoles.** De Waard (7) stated that the evaluation of fungal resistance to DMI fungicides is difficult since the resistance level is frequently low and its development can only be detected when there is preliminary (baseline) data which can be used as reference IC_{50}.

Data reported by Stolte (22) for tebuconazole, IC_{50} < 0.1 for 0.32mg/L, and for cyproconazole, IC_{50} < 0.1 for 0.53 mg/L, differed from our data, which included IC_{50} higher than those reported by that author, suggesting sensitive reduction of *Dtr* to DMI fungicides. That same author, conducting tests to monitor *in vitro* the mycelial sensitivity of *Dtr* isolates from wheat to fungicides in 2005, determined IC_{50} < 1mg/L for cyproconazole, epoxiconazole, propiconazole and tebuconazole. She considered the isolates were sensitive to these active ingredients. Beard et al. (3), in a sensitivity study conducted with *Dtr*, reported IC_{50} of 0.19 mg/L for epoxiconazole, 0.39 mg/L for propiconazole, and 0.25 mg/L for tebuconazole. Hunger & Brown (13), working with *Dtr,* obtained IC_{50} of 0.04 mg/L for propiconazole and 0.19 mg/L for tebuconazole. The IC_{50} for propiconazole found in our study ranged from 0.49 to 1.28 mg/L, while for tebuconazole it varied from

Table 4. Sensitivity reduction factor (SRF) of *Drechslera tritici-repentis*, isolated from wheat, to DMI fungicides

Fungicide	Isolates					Mean
	01/QTZ	02/ONX	03/HZT	04/GUA	05/CD	
Cyproconazole	-*	-	-	-	-	-
Epoxiconazole	3.91	1.00	3.17	1.80	1.28	2.23
Propiconazole	1.75	2.61	1.00	1.02	2.38	1.75
Prothioconazole	2.00	1.77	2.33	1.00	1.89	1.80
Tebuconazole	1.30	1.66	1.29	1.00	1.57	1.36
Mean	2.24	1.61	1.95	1.20	1.66	

* Insensitive.

1.41 to 2.34 mg/L. Comparing our values with those reported by Hunger & Brown (13), IC_{50} has increased 12-32 times for propiconazole and 7-12 times for tebuconazole. The IC_{50} values determined in our study were superior to those found by other authors, indicating reduction in the sensitivity of *Dtr* to DMI.

Drechslera tritici-repentis sensitivity to strobilurins. QoI fungicides interfere with mitochondrial respiration by blocking electron transfer by the cytochrome bc1 complex, which interferes with ATP formation (Anesiadis). According to Anesiadis et al. (1), strobilurins act on the leaf surface, inhibiting the early stages of the infection process, such as spore germination, initial establishment and penetration of the pathogen.

Fungicides belonging to the same chemical group can show cross-resistance. This means that a fungicide resistant to an isolate can also be resistant to another fungicide that has the same biochemical mode of action (4, 5, 6, 7, 9). This fact was not proven in this study because there was differential interaction.

The sensitivity behavior among isolates to fungicides showed reduction/loss for QoI, evidencing that the selection pressure exerted by the continuous use of fungicides to control diseases in wheat crop may have led to a shift in sensitivity of the pathogen population to QoI fungicides. Rodrigues et al. (24) argued that, for strobilurins, there may be cross-resistance to azoxystrobin, pyraclostrobin and trifloxystrobin. Nevertheless, we do not have any explanation for the performance of pyraclostrobin, the most efficient fungicide for spore germination inhibition.

Dtr isolates showed less sensitivity (IC_{50} > 40 mg/L) to azoxystrobin, kresoxim-methyl, trifloxystrobin and picoxystrobin in relation to spore germination. It is not known whether there was a shift because the baseline was not established when the individuals of the fungal population were still sensitive.

Even using only five isolates, we have shown differences in sensitivity among fungal strains and fungicide power. When a large number of fungicides and concentrations are used, the number of isolates has to be reduced. Thus, in the next step of this study, the number of isolates will be increased by using the collection of *Dtr* and *D. siccans* isolates (Tonin, Reis, Danelli) stored in the mycology collection of the Laboratory of Plant Pathology - Mycology, University of Passo Fundo, to track fungal sensitivity to only two fungicides, azoxystrobin and pyraclostrobin.

The use of fungicides must be managed according to anti-resistance strategies. These strategies are based on the principle that when there is fungicide application, a selection pressure is exerted on the pathogen population and can, in the long or short term, depending on the genetic mechanisms involved, result in the selection and predominance of the less sensitive individuals in the fungal population (11).

In the 2005/06 wheat growing season, after 20 years of DMI use, the first complaint of YLS control failure in Brazil was recorded (26).

According to our results, some *Dtr* isolates had a reduction in sensitivity to triazole fungicides but at different degrees. The fungicides tested in our study belong to the same family of triazoles and have the same mechanism of action, DMI (9). However, their potency differed among the representatives of the group. The fact of belonging to the same family did not assure that they had the same fungitoxicity.

The fungicide prothioconazole was most potent in inhibiting *Dtr* mycelial growth. Cyproconazole showed the highest IC_{50} values among the tested triazoles and was considered least fungitoxic.

Our results confirm the hypothesis that the failure to control YLS in wheat, observed in the last seasons, can be attributed to a reduction in the fungal sensitivity to the fungicides used to control the disease.

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