LUIS IGNACIO CAZÓN

SENSITIVITY TO FUNGICIDES IN *Pyricularia* POPULATIONS FROM WHEAT AND SIGNAL GRASS IN MINAS GERAIS

Thesis submitted to the Phytopathology Graduate Program of the Universidade Federal de Viçosa, in partial fulfillment of the requirements for the degree of *Doctor Scientiae.*

Adviser: Emerson Medeiros Del Ponte

VIÇOSA - MINAS GERAIS
2021

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
C386s
2022

Cazón, Luis Ignacio, 1982-
Sensitivity to fungicides in Pyricularia populations from wheat and signal grass in Minas Gerais / Luis Ignacio Cazón. – Viçosa, MG, 2022.
1 tese eletrônica (102 f.): il. (algumas color.).

Texto em inglês.
Inclui apêndices.
Orientador: Emerson Medeiros del Ponte.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Fitopatologia, 2022.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2022.110
Modo de acesso: World Wide Web.

1. Brusone - Epidemiologia. 2. Trigo - Doenças e pragas - Controle. 3. Resistência aos fungicidas. 4. *Magnaporthe oryzae*. 5. *Pyricularia oryzae*. I. Ponte, Emerson Medeiros del, 1973-. II. Universidade Federal de Viçosa. Departamento de Fitopatologia. Programa de Pós-Graduação em Fitopatologia. III. Título.

CDD 22. ed. 632.46

Bibliotecário(a) responsável: Alice Regina Pinto CRB6 2523

LUIS IGNACIO CAZÓN

SENSITIVITY TO FUNGICIDES IN *Pyricularia* POPULATIONS FROM WHEAT AND SIGNAL GRASS IN MINAS GERAIS

Thesis submitted to the Phytopathology Graduate Program of the Universidade Federal de Viçosa, in partial fulfillment of the requirements for the degree of *Doctor Scientiae.*

APPROVED: February 24, 2022

Assent:

Luis Ignacio Cazón
Author

Emerson Medeiros Del Ponte
Adviser

To Laura, Olivia e Alma,
To Lucho, Miriam and Gloria,
To Bruno, Andy, Caro, Flavia and Julia

ACKNOWLEDGMENTS

My partner Laura for her constant support, motivation and understanding at this stage of our lives. Without you, this achievement would not have been possible.

To my daughters Alma and Olivia, for understanding my absences, for relieving the bad days with kisses and hugs and for always being my biggest motivation.

To my parents Luis and Miriam, my aunt Gloria, to my brothers Bruno, Andy, Caro, Flavia and Julia for always being there with the right words in difficult times.

To my adviser, Professor Emerson Del Ponte, for giving me the opportunity to be part of this working group, for always encouraging me to get out of my comfort zone, for the great professional and personal respect in the monitoring of this thesis work.

To Alejandro Rago, for encouraging and motivating me to live this great professional and personal experience outside Argentina.

To Professor Sérgio Brommonschenkel, for the co-supervision of this work.

To my laboratory friends, João, Gustavo, Kaique, Jhonatan, Maíra, Sheila, Bianca, Glaucia, Renato, Henrique and Carol. For being an emotional and professional support during these years in Brazil. They are people I will always carry in my heart.

To Sara, for her availability in helping me to resolve administrative issues related to the Graduate Program.

Débora, Maria Laura, Nathália, Alvaro and Monalisa for their help in the execution and evaluation of the experiments.

Deivid, for his help in the design of the primers.

Mariela, for her help in processing the DNA sequences.

To all colleagues from the DFP Graduate Program, who contributed directly and indirectly to my professional training.

To the Universidade Federal de Viçosa, the Department of Plant Pathology and the Graduate Program in Plant Pathology.

To the Instituto Nacional de Tecnología Agropecuaria (INTA) for the financial support during these 4 years outside Argentina.

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq, for the scholarship.

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

To all the Professors of the Department of Phytopathology - UFV, for their generosity in transmitting their knowledge, inside and outside the classroom.

To Professor Eduardo Mizubuti and members of the BIOPOP Laboratory, for their contribution with the infrastructure for the development of molecular techniques.

To Daniel and Delfim, for their help in the *in vivo* experiments.

To all those friends who have always been an emotional support for me and my family in this wonderful experience in Brazil. I dare not mention them all for fear of forgetting any of them. Thank you very much!

ABSTRACT

CAZÓN, Luis Ignacio, D.Sc., Universidade Federal de Viçosa, February, 2022. **Sensitivity to fungicides in *Pyricularia* populations from wheat and signal grass in Minas Gerais**. Adviser: Emerson Medeiros Del Ponte.

Wheat blast, caused by *Pyricularia oryzae Triticum* pathotype, is a damaging disease of wheat in the Brazilian Cerrado. Fungicides play an important role in management, but their efficacy has been variable. Failures in disease control have been associated with the presence of fungicide resistant populations. To determine if these resistance patterns are also present in *Pyricularia* populations infecting wheat and signal grass from MG state, we characterized phenotypically and genotypically the resistance of the blast pathogen against seven fungicides belonging to three chemical groups (DMI, QoI and SDHI). The *in vitro* sensitivity was assessed in 64 isolates obtained from the Triângulo Mineiro and Sul de Minas. For DMIs, the EC_{50} was estimated for all isolates. For QoIs and SDHIs, the EC_{50} was obtained for 13 isolates prior to determining a discriminatory dose. Control efficacy of each fungicide was also evaluated *in vivo* upon inoculation of wheat heads with a resistant (R) or a sensitive (S) PoT isolate (determined *in vitro*) to each fungicide. The presence of mutations in the genes encoding the target proteins of the different groups of fungicides was also evaluated. For the DMIs (TEBU - tebuconazole and EPOX - epoxiconazole), despite showing relatively high EC_{50} values for some isolates, the control efficacy on wheat heads was high. We found no association between mutations in the *cyp51A* region and resistance to DMI. For QoIs, azoxystrobin (AZOX) did not inhibit the *in vitro* germination of PoT even with an extra dose of 100 µg/ml, and was poorly controlled *in vivo* (~ 20%). For pyraclostrobin (PYRA), despite the presence of isolates with high leves of spore germination *in vitro* using a discriminatory dose of 1.3 µg/ml, the control efficacy was also high. The G143A substitution was present in all PoT *cytB* sequences, even in those of the PYRA sensitive isolates. The non-PoT isolates did not present mutations in the *cytB* hot spots. For SDHIs, the most fungitoxic in *in vitro* and *in vivo* experiments was benzovindiflupyr, followed by bixafen and fluxapyroxad. Molecular analysis of the sdh subunits showed no relationship between mutations and sensitivity to SDHI fungicides. For the seven fungicides tested in this work, the PoT isolates were statistically less sensitive than the isolates from signal grass. Our

results demonstrate that PoT populations can be controlled *in vivo* with at least one fungicide of each of the three chemical groups evaluated. Contrary to previous statements, determining EC_{50} values in *in vitro* experiments was not sufficient to infer loss of efficacy in *in vivo* experiments. Benzovindiflupyr was the most effective active ingredient evaluated in this work. Conversely, azoxystrobin should not be considered for controlling wheat blast.

Keywords: Wheat blast. Fungicide resistance. Epidemiology. *Magnaporthe oryzae*. *Pyricularia oryzae.*

RESUMO

CAZÓN, Luis Ignacio, D.Sc., Universidade Federal de Viçosa, fevereiro de 2022. **Sensibilidade a fungicidas em populações de *Pyricularia* de trigo e braquiária em Minas Gerais.** Orientador: Emerson Medeiros Del Ponte.

A brusone do trigo, causada por *Pyricularia oryzae* patótipo *Triticum*, é uma doença importante para o trigo no Cerrado Brasileiro. O uso de fungicidas no manejo é importante, mas os resultados têm sido variáveis. Falhas no controle da doença têm sido associadas a populações resistentes a fungicidas. Para determinar se esses padrões de resistência também estão presentes em populações de *Pyricularia* que infectam trigo e braquiária no estado de MG, caracterizamos fenotípica e genotipicamente a resistência do patógeno da brusone contra sete fungicidas pertencentes a três grupos químicos (DMI, QoI e SDHI). A sensibilidade *in vitro* foi avaliada em 64 isolados provenientes do Triângulo Mineiro e Sul de Minas. Para os DMIs, a EC_{50} foi estimada para todos os isolados. Para QoIs e SDHIs, o EC_{50} foi obtido para 13 isolados, antes de determinar uma dose discriminatória. A eficácia de controle de cada fungicida também foi avaliada *in vivo* após inoculações em espigas com isolado PoT resistente (R) e sensível (S) (determinados *in vitro*) para cada fungicida. Mutações nos genes que codificam proteínas alvo dos diferentes grupos de fungicidas também foram avaliadas. Para os DMIs (TEBU - tebuconazol e EPOX - epoxiconazol), apesar de apresentar valores de EC_{50} relativamente elevados para alguns isolados, as eficácias do controle em espiga foram elevadas. Não encontramos associação entre mutações na região *cyp51A* e resistência aos DMIs. Para QoIs, azoxistrobina (AZOX) não inibiu a germinação *in vitro* de PoT mesmo em doses de 100 µg/ml, e o controle *in vivo* foi baixo (~ 20%). Para a piraclostrobina (PYRA), apesar da presença de isolados com altos níveis de germinação *in vitro* de esporos com dose discriminatória de 1,3 µg/ml, as eficácias de controle também foram elevadas. A mutação G143A esteve presente em todas as sequências *cytB* dos isolados PoT, mesmo naquelas dos isolados sensíveis a PYRA. Os isolados não-PoT não apresentaram mutações nos hot spots *cytB*. Dentre os SDHIs, o fungicida mais fungitóxico *in vitro* e *in vivo* foi o benzovindiflupir (BENZ), seguido por bixafen (BIXA) e fluxapiroxade (FLUX). Não houve relação entre mutações nas subunidades *sdh* e sensibilidade aos fungicidas SDHI. Para os sete fungicidas

testados neste trabalho, os isolados PoT foram estatisticamente menos sensíveis que os não-PoT. Nossos resultados demonstram que as populações de PoT podem ser controladas *in vivo* com pelo menos um fungicida de cada um dos três grupos químicos avaliados. Ao contrário dos anteriores relatos, a determinação dos valores de EC_{50} em experimentos *in vitro* não foi informativa para inferir perda de eficácia *in vivo* dos fungicidas. O benzovindiflupir foi o ingrediente ativo mais efetivo avaliado neste trabalho. Por outro lado, a azoxistrobina não deve ser considerada no controle da doença.

Palavras-chave: Brusone do trigo. Resistência a fungicidas. Epidemiologia. *Magnaporthe oryzae*. *Pyricularia oryzae.*

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF ACRONYMS AND ABBREVIATIONS

AZOX	Azoxystrobin
BENZ	Benzovindiflupyr
BIXA	Bixafen
DMI	Demethylation Inhibitor
DNA	Deoxyribonucleic Acid
EC_{50}	Effective Concentration 50
EPOX	Epoxiconazole
FLUX	Fluxapyroxade
FRAC	Fungicide Resistance ActionCommittee
ha	Hectare
MG	MInas Gerais
mL	Milliliter
PCR	Polymerase Chain Reaction
PoT	*Pyricularia oryzae Triticum* Pathotype
PYRA	Pyraclostrobin
QoI	Quinone Outside Inhibitor
SDHI	Succinate Dehydrogenase Inhibitors
TEBU	Tebuconazole
TM	Triângulo Mineiro

LIST OF SYMBOLS

%	Percentage
^{o}C	Celsius Degree
µg	Microgram
ρ	Pearson's Correlation Coefficient

SUMMARY

1 INTRODUCTION

1.1 Distribution and significance of wheat blast

Wheat blast, caused by the ascomycete fungus *Pyricularia oryzae* Cavara, *Triticum* pathotype (PoT) (Cruz & Valent, 2017), was firstly observed in 1985 in wheat fields grown in Paraná state, Brazil (Igarashi et al. 1986), from where it further spread to all wheat regions in the country (Igarashi *et al.*, 1986; Goulart & Paiva, 1990; Prabhu *et al.*, 1992; Anjos *et al.*, 1996; Ceresini *et al.*, 2018). The first report outside Brazil occurred a decade later (1996) in the department of Santa Cruz, eastern Bolivia (Barea & Toledo, 1996). In 2002, wheat blast was reported in Paraguay (Viedma, 2005), and in 2007 the disease was reported in Northern Argentina (Cabrera & Gutierrez, 2007; Alberione *et al.*, 2008; Perelló *et al.*, 2015). The first intercontinental jump occurred in 2016 when the disease arrived in Bangladesh, South Asia (Callaway, 2016; Islam *et al.*, 2019). Recently, the disease was detected in Zambia, Africa, in 2017 (Tembo, 2019; Tembo *et al.*, 2020).

Despite its occurrence in a wide range of latitudes, wheat blast is most common and more damaging in the tropical (warmer) climate conditions. Temperatures as high as 25 to 30^{o}C associated with frequent leaf wetness periods (24 to 40h) favor disease infection (Cardoso *et al.*, 2008). Under such conditions, epidemics may occur on both the leaves and heads and lead to severe yield losses, but the head blast is much more common (Cruz *et al.*, 2016b; Gongora-Canul *et al.*, 2020). In fact, significant yield losses have been associated more often with wheat head blast rather than leaf blast (Cruz & Valent, 2017). The disease reduces grain yield and quality, producing small, shriveled and deformed grains. Under conducive conditions, yield losses in susceptible varieties can reach severe levels as high as

70% (Goulart & Paiva, 2000; Pagani *et al.*, 2014; Santana *et al.*, 2016; Valent *et al.*, 2021).

In Brazil, wheat blast is currently a limitation to the expansion of wheat in the states of Goiás and Minas Gerais; the largest wheat-producing states in the tropics (Oliveira *et al.*, 2015; Ceresini *et al.*, 2018). Currently, breeding programs are focusing their attention on the development of new cultivars that are more adapted to the Brazilian Cerrado conditions due to grain quality of the produce and proximity to major consumers. A current limitation for expanding the production in the region is the high conducive conditions for wheat blast epidemics, and the difficulty for obtaining cultivars with durable resistance for the disease (Maciel, 2011; Ceresini *et al.*, 2019).

1.2 Symptoms, biology and epidemiology

The wheat blast pathogen can infect other parts of the plant besides the wheat leaves and heads including the peduncle and seeds (Urashima *et al.*, 2009; Cruz & Valent, 2017; Singh *et al.*, 2021). Leaf lesions are typically eye-spotted or spindle-shaped with light tan centers, often with dark brown margins and/or chlorotic halos. These lesions can range from 1mm to 10 mm in size depending on the aggressiveness of the strain and susceptibility of the cultivar (Valent *et al.*, 2021) (Fig. 1A). During severe epidemics, those lesions may coalesce and affect the entire leaf. Under wet conditions, the lesions are capable of sporulating from the clear center turning it gray, due to the formation of conidia and conidiophores (Ceresini *et al.*, 2018, 2019; Valent *et al.*, 2021) (Fig. 1B). The most distinguishable symptom is observed on the head. Depending on the point of infection, partial or total bleached heads could be observed (Igarashi *et al.*, 1986; Cruz & Valent, 2017; Singh *et al.*,

2021) (Fig. 1C, D). The pathogen blocks the transport of nutrients, affecting the weight and quality of the grain above the point of infection (Goulart *et al.*, 1990; Urashima *et al.*, 2009; Ceresini *et al.*, 2019). In susceptible cultivars, a dark gray mass of spores could be observed on the rachis where the infection occurred (Islam *et al.*, 2016; Singh *et al.*, 2021) (Fig. 1E).

The wheat blast pathogen is capable of infecting several cereal and forage crops, as well as several grasses, such as *Panicum maximum, P. miliaceum, Eleusine coracana, Avena sativa, Lolium perenne, Stenotaphrum secundatum, Rhynchelytrum roseum, U. plantaginea* and *U. brizantha* (Urashima *et al.*, 1993, 2017; Oh *et al.*, 2002; Tosa *et al.*, 2004, 2006; Couch *et al.*, 2005; Hirata *et al.*, 2007; Tosa & Chuma, 2014; Farman *et al.*, 2017; Cruz & Valent, 2017; Singh *et al.*, 2021; Pak *et al.*, 2021). Some authors affirm that the disease risk increases if those alternative hosts are cultivated in large geographical areas, which is the case of signal grass (Fig. 1F, G), particularly U. *brizantha* (Jank *et al.*, 2014), that is grown as a forage crop in more than 90 million hectares in Brazil and it was reported by as the main source of inoculum for the occurrence of wheat blast (Maciel *et al.*, 2014; Castroagudín *et al.*, 2016; Ceresini *et al.*, 2019). Preliminary studies in our laboratory suggest that infections in wheat and signal grass are caused by different pathotypes (Ascari, 2021), so if so, the response to the management strategies implemented (chemical control for example) would also be different.

Figure 1. Typical wheat blast symptoms. (A) Wheat leaf blast symptoms in the field. (B) Sporulating lesions after leaf incubation under moist and observed under the magnifying glass. (C) Heads totally bleached by PoT infection in the field. (D) Typical partial infected head. (E) Dark gray spore mass of PoT on the rachis. (F) Leaf blast symptoms on *Urochloa brizantha* and (G) and *Urochloa plantaginea*.

Senescent plant tissues may also contribute to inoculum survival during the off-season for at least five months in wheat-infected residues (Pizolotto *et al.*, 2019). Spores produced on alternative hosts or crop residues can disperse through wind across fields distanced at least 1 km from the inoculum source (Urashima *et al.*, 2007). The amount of spores in the air depends on the environmental conditions. For temperatures between 25°C and 28°C, relative humidity of 76% to 100%, and light rain (< 5 mm) the amount of spore in the air is increased (Leach, 1980; Danelli *et al.*,

2019), and after a period of heavy rains the number of spores decreases (Silva & Prabhu, 2005; Danelli *et al.*, 2019). As to long distance dispersal, infected seeds or grains are the most likely source of inoculum for disease establishment (Tanaka *et al.*, 2009; Gomes *et al.*, 2018). In fact, genome analyse on isolates from Bangladesh indicated a high similarity with isolates from South America (Islam *et al.*, 2016; Malaker *et al.*, 2016), suggesting that the pathogen was likely introduced via contaminated grains imported from this region (Valent *et al.*, 2021). When blast-infected seeds were sown, fungal sporulation has been observed on the lower senescent wheat leaves in the field (Cruz *et al.*, 2015a). Secondary infection in the field could be due to inoculum produced within the canopy and by airborne spores from an external source (Gongora-Canul *et al.*, 2020).

As mentioned, temperatures between 25ºC and 30ºC associated with frequent leaf wetness periods (24 to 40h) favor disease infection (Cardoso *et al.*, 2008). Severe blast epidemics in South American countries coincided with El Niño years (Kohli *et al.*, 2011; Singh *et al.*, 2021; Valent *et al.*, 2021). Many days of continuous rain and high temperatures favor spore deposition on the head. Spores stick tightly on the cuticle through an adhesive mucilage composed of glycerol (Ebbole, 2007; Wilson & Talbot, 2009). The free water stimulates the formation and accumulation of pressure in the appressorium, which starts the process of penetration on the host's surface 12 hours later (Cruz *et al.*, 2016d). This process is favored by a melanin layer that is produced and deposited in the germ tube to support a turgor pressure (Boddy, 2016; Fernandez & Orth, 2018). After entering the germ tube into the plant tissue, hyphae move across cells through plasmodesmata (Ebbole, 2007; Wilson & Talbot, 2009; Boddy, 2016; Fernandez & Orth, 2018). In compatible interactions, visible symptoms on leaf and heads begin from 72 to 120 hours and the sporulation process

in the necrotic lesions begin over 120 hours later (Cruz *et al.*, 2015b, 2016d). This cycle can be repeated many times during the crop cycle in the field, which is characteristic of polycyclic epidemics (Gomes *et al.*, 2019; Gongora-Canul *et al.*, 2020; Mills *et al.*, 2020).

1.3 Disease management

Efficient and economic management programs targeting wheat blast disease are not yet available and current solutions seem to be region-specific (Cruz *et al.*, 2019; Valent *et al.*, 2021). Shifts in sowing dates may be useful as an escape measure in the tropical growing region in Brazil. Indeed, a two to three month delay (from March to May) in the sowing date drastically reduced disease intensity in the Triangulo Mineiro growing region of Brazil under irrigation (Coelho *et al.*, 2016). Other cultural practices include the use of certified seeds to avoid pathogen introduction into disease-free areas and the non-use of alternative hosts in crop rotation schemes (Gomes *et al.*, 2018; Ceresini *et al.*, 2019; Martinez *et al.*, 2021; Singh *et al.*, 2021).

Fertilization strategies can affect PoT infection processes in wheat. Many studies suggest that nutrients such as silicon (Si), zinc (Zn), calcium (Ca) and magnesium (Mg) can help to reducing the intensity of wheat blast (Xavier Filha *et al.*, 2011; Debona *et al.*, 2014, 2016, 2017; Rios *et al.*, 2017; Rodrigues *et al.*, 2017; Aucique-Pérez *et al.*, 2017, 2020; Surovy *et al.*, 2020; Moreira *et al.*, 2020). The effect of the nitrogen fertilization is still not clear in the interaction of wheat-PoT, but some studies report a positive correlation between nitrogen rates and disease intensity (Huber & Thompson, 2007; Silva *et al.*, 2019).

The use of genetic sources for resistance against PoT is hampered by pathogen variability and the conduciveness of the environment where wheat blast

epidemics are most frequent - the tropics (Cruz *et al.*, 2010; Rocha *et al.*, 2019; Valent *et al.*, 2021). Currently, some cultivars exhibit partial resistance against PoT, which was associated with the presence of the 2NS chromosomal segment translocated from *Aegilops ventricosa* (Cruz *et al.*, 2010). The use of these cultivars is a more effective strategy under conditions less favorable for wheat blast epidemics (Cruz *et al.*, 2016c; Jéssica Rosset Ferreira *et al.*, 2018; Cardozo Téllez *et al.*, 2019; Cruppe *et al.*, 2020). In addition, leaf blast resistance has shown an inconsistent relationship with head blast resistance, indicating that leaf resistance may not be a good predictor of head blast resistance (Roy *et al.*, 2021). As a last resort, fungicide applications have been used to control the disease but the results have been variable and levels of control generally modest (Cruz *et al.*, 2019; Ascari *et al.*, 2021).

1.3.1 Chemical control

The efficacy of fungicide sprays for controlling wheat blast depends on several factors such as the active ingredient, application timing, environment and the degree of host resistance (Cruz *et al.*, 2011a; Ceresini *et al.*, 2019; Ascari *et al.*, 2021). Seed treatment with fungicides (e.g. benomyl, difenoconazole and carboxin+thiram) is recommended to avoid infection in cotyledons and primary leaves (Sadat & Choi, 2017; Yesmin *et al.*, 2020; Singh *et al.*, 2021). Fungicides are usually applied at the heading stage and sequential sprays depending on the disease risk (Ceresini *et al.*, 2018). Currently, 57 commercial fungicides have been registered for wheat blast control in Brazil (AGROFIT, 2021). Most of the commercial formulations include quinone outside inhibitor (QoI) and demethylation inhibitor (DMI) fungicides, marketed solely or as premixes (Ceresini *et al.*, 2019; Valent *et al.*, 2021; Ascari *et al.*, 2021).

In less susceptible cultivars, control efficacy levels were reported as high as 90% using two applications of QoI+DMI premix (Rios *et al.*, 2016). However, even four sprays were not capable of completely controlling wheat blast in susceptible cultivars grown at disease-conducive environments (Urashima *et al.*, 2017; Singh *et al.*, 2021). A recent meta-analytic study showed the effectiveness of DMIs applied solely or as premix with QoI. Efficacies greater than 50%, on average, were obtained with the use of trifloxystrobin + prothioconazole, tebuconazole, azoxystrobin + tebuconazole, and trifloxystrobin + prothioconazole. An economic analysis in that study showed lower risk of economic loss when a multisite (mancozeb) was compared with the site-specific premixes, which were less effective in the tropics compared with the subtropics (Ascari *et al.*, 2021).

In recent years, a succinate dehydrogenase inhibitor (SDHI), mainly the second generation carboxamides, has been introduced in combination with QoI and/or DMI for managing wheat diseases (AGROFIT, 2021). None of these products are currently labeled for wheat blast, but they are used for managing foliar and head diseases of wheat. Finally, the difficulty for the sprays to reach and fully cover the target site (rachis) is acknowledged as an important factor affecting fungicide efficacy (Valent *et al.*, 2021). Infection can occur at any position of the rachis, but greater yield losses occur when it's happening at the base, because this leads to the complete head bleaching (Goulart *et al.*, 2007; Kohli *et al.*, 2011; Cruz *et al.*, 2011b). The presence of the spikelets makes it difficult for the fungicide suspension to reach the rachis (Ceresini *et al.*, 2019).

1.4 Fungicide resistance in wheat blast

The historical use of DMIs and QoIs fungicides, and the recent introduction of SDHIs, to manage wheat diseases (Tormen *et al.*, 2013; Rios *et al.*, 2016; Ceresini *et al.*, 2018, 2019; AGROFIT, 2021) has been suggested as a factor contributing to the general low efficacy of most fungicides due to fungicide resistance (Dorigan *et al.*, 2019; Poloni *et al.*, 2021; Vicentini *et al.*, 2021). In the last years, several research has shown *P. oryze* (either infecting rice or wheat) populations that exhibited some level (sometimes increasing) of resistance to DMIs (tebuconazole and epoxiconazole) (Ceresini *et al.*, 2018; Dorigan *et al.*, 2019; Poloni *et al.*, 2021; D'Ávila *et al.*, 2021) and QoIs (azoxystrobin and pyraclostrobin) (Castroagudín *et al.*, 2015; Oliveira *et al.*, 2015; D'Ávila *et al.*, 2021). For SDHI fungicides, a recent study reported resistance to fluxapyroxad in wheat-infecting *P. oryzae* populations collected in 2012, even before the use of SDHI fungicides, suggesting the existence of an intrinsic resistance in the pathogen population (Vicentini *et al.*, 2021). A common feature in the aforementioned works is that the report of PoT resistant populations was made based on the *in vitro* response (EC_{50} values) of the isolates without the use of *in vivo* tests using commercial doses. According to Reis et al. (2015) field situations and *in vitro* responses could be different. These aspects have not yet been studied for this fungus. In a recent review, Valent et al. (2021) highlights the importance of proper monitoring of the sensitivity of fungal populations.

1.5.1 Fungicide resistance mechanisms

Fungicide resistance could be related with different mechanisms: (1) mutations in genes that encode the target protein resulting in decreased protein affinity to the

inhibitors, (2) overexpression of that gene, resulting in a higher amount of target protein, (3) increase the efflux transporters which prevent the accumulation of toxic compounds inside the fungi cells and (4) activation of an alternative respiration route, which is more relevant for QoI and SDHI resistance (Leroux & Walker, 2011; Hollomon, 2015; Yamashita & Fraaije, 2018; Dorigan *et al.*, 2019). The most common mechanism is the modification of the target protein via point mutations (Ishii & Hollomon, 2015). Triazoles, for instance, belong to the sterol demethylation inhibitor (DMI) group. The target of these fungicides is the 14-alpha-demethylase enzyme encoded by the *cyp51* gene. This enzyme participates in the formation of the cell membrane by catalyzing the synthesis of sterol. There are several studies that link variations in this gene with different responses against this chemical group (Wyand & Brown, 2005; Leroux *et al.*, 2007; Leroux & Walker, 2011; Kikuhara *et al.*, 2019; Dorigan *et al.*, 2019; Mair *et al.*, 2019; Tucker *et al.*, 2020; de Ramón-Carbonell & Sánchez-Torres, 2020; Poloni *et al.*, 2021). In the case of QoI and SDHI, the target sites are in the respiration chain. Strobilurin are considered Quinone-outside inhibitors (QoI). Different mutations in cytochrome b gene (*cytB*) are reported as responsible for resistance (Castroagudín *et al.*, 2015; Ceresini *et al.*, 2019; Valent *et al.*, 2021; Vicentini *et al.*, 2021). The most important is the G143A, which leads to an interference with the linker part of QoI molecules resulting in strikingly reduced binding (Kim *et al.*, 2003; Hollomon, 2015). Carboxamides are considered succinate dehydrogenase inhibitors (SDHI). Those molecules specifically bind to the ubiquinone binding site (Q site) of mitochondrial complex II, thus inhibiting fungal respiration (Avenot et al. 2010). Complex II is a membrane-anchored protein and represents a link between mitochondrial respiration and the Krebs cycle (Cecchini, 2003; Ishii & Hollomon, 2015). It consists of four subunits: *sdhA*, *sdhB*, *sdhC*, and

sdhD. The SDHI binding site in the protein complex is composed of subunits B, C and D. Therefore, it is suggested that mutations in genes encoding any subunit could modify the configuration of the protein conferring resistance (Avenot & Michailides, 2010; Leroux *et al.*, 2010; Amiri *et al.*, 2014; Yamashita & Fraaije, 2018).

1.7 Hypothesis and objectives

In relation to previous studies that suggest that the causal agent of wheat blast corresponds to a different pathotype than that of signal grass, our first hypothesis is that the isolates from wheat have a lower sensitivity to fungicides than the isolates from signal grass due to their increased fungicide exposition. Furthermore, since previous studies did not include *in vivo* experiments with commercial doses of fungicides, our second hypothesis is that there are chemical alternatives within the main chemical groups for an effective control of wheat blast.

The isolates studied were collected in Minas Gerais (where epidemics are more severe) in 2018 and 2019 from wheat and other grasses, mainly signal grass (*Urochloa brizantha*). Seven fungicides, belonging to three chemical groups, were evaluated: tebuconazole and epoxiconazole (DMIs), azoxystrobin and pyraclostrobin (QoIs), bixafen, fluxapyroxad and benzovindiflupyr (SDHIs).

The objectives of this work were: 1) to characterize the *in vitro* sensitivity to seven fungicides for a representative group of PoT lineage as well as the lineages infecting signal grass in the Minas Gerais growing region; 2) to identify the variations in the genes that encode the target sites of the different group of fungicides; 3) to determine the *in vivo* response to fungicides in wheat heads inoculated with a respective resistant and sensitive isolate according to *in vitro* screening.

2 MATERIALS AND METHODS

2.1 Collection of isolates

A total of 64 isolates, collected in 2018 and 2019 in Minas Gerais, were sourced from a larger collection of six hundred isolates obtained in a previous study. Two different regions in MG state were considered when selecting the isolates. Forty isolates were selected (24 PoT and 16 non-PoT isolates) from the "Triangulo Mineiro" and the 24 remaining isolates were collected in the "Centro Sul" region (16 PoT and 8 non-PoT isolates) (Fig. 2). The lineage of the isolates were characterized in a previous work based on host of origin (wheat or signal grass) and PCR reaction using the PoT-specific primer C17 (Thierry *et al.*, 2020). Reactions of C17 (+) are suggestive of PoT while C17 (-) indicate a grass lineage (Ascari, 2021).

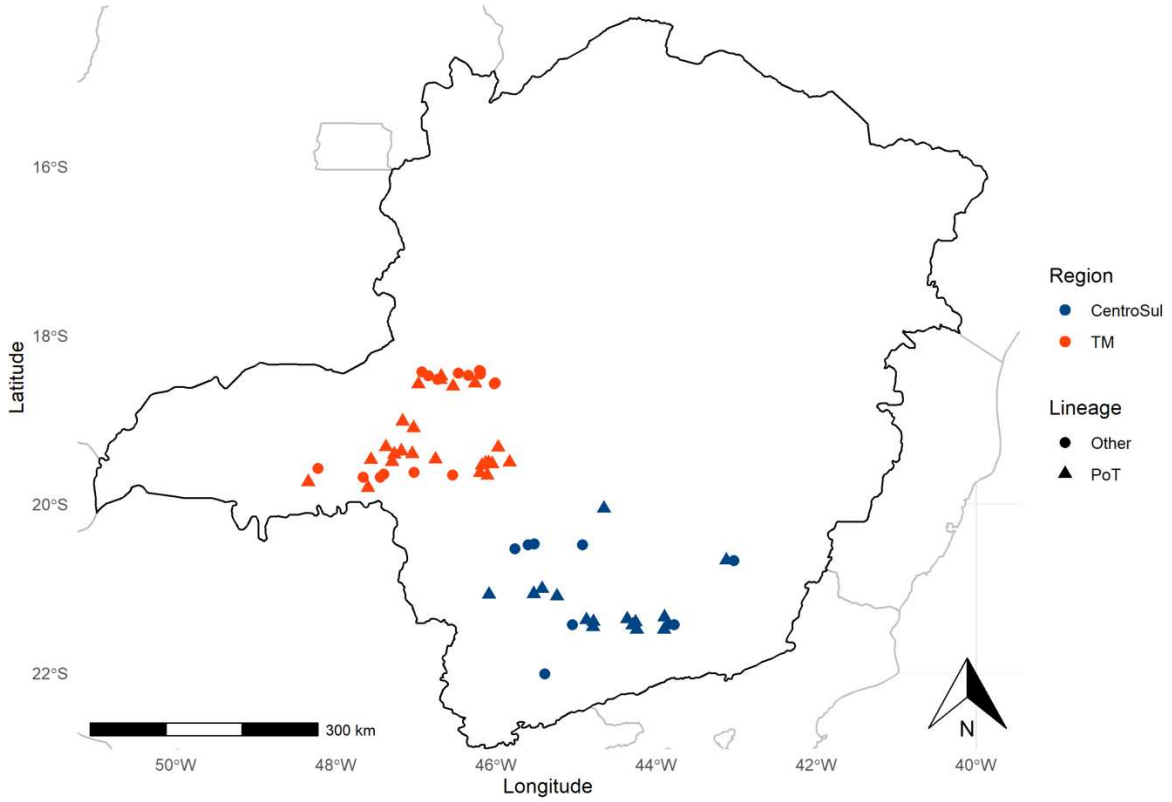

Figure 2. Map of Minas Gerais depicting the different locations and lineages of the Po isolates characterized in this work. Different colors represent different regions (● = Centro-Sul de Minas, ● = Triângulo Mineiro/Alto Paranaíba). Different shapes represent lineages (▲ = *P. oryzae Triticum* lineage, ● = grass lineages).

2.2 *In vitro* sensitivity assay for DMI fungicides

A piece of filter paper that preserved the isolate was recovered from the -10°C long-term storage and reactivated on Potato Dextrose Agar (PDA). A 7-day-old mycelial 8-mm-plugs were transferred to 9 cm - petri dishes supplemented with chloramphenicol and streptomycin (100 µg/mL) and increasing concentrations of the tebuconazole and epoxiconazole fungicides (0.0, 0.5, 1.0, 2.0, 3.0 and 5.0 µg/mL). The fungicides were diluted from the commercial products Tebufort - UPL (tebuconazole 200 g/L) and Rubric - FMC (epoxiconazole 125 g/L). Each concentration was tested in three replicates for each isolate. The dishes were incubated in a grown chamber with controlled temperature of 25°C (±2°), and a

photoperiod of 12/12 hours (light/darkness) (Cruz *et al.*, 2016a; Urashima *et al.*, 2017). After nine days, the colony diameters were measured with a digital caliper and the 8 mm diameter of the original mycelial plug subtracted. The effective concentration leading to 50% inhibition of mycelial growth (EC_{50}) was estimated for each isolate. The experiment was performed twice.

2.3 *In vitro* sensitivity assays for SDHI and QoI fungicides

For this purpose, the experiments were conducted in two steps. First, the EC_{50} of 13 representative isolates from the study collection was calculated. Subsequently, based on the obtained values, a discriminatory dose was estimated for each fungicide and applied to all collection (FRAC-BR, 2021, Sautua & Carmona, 2021). The experiments were performed twice.

2.3.1 Spore production.

A piece of paper preserving each isolate was recovered from -10°C storage and re-activated on Potato Dextrose Agar (PDA). A 5-day-old mycelial plug was transferred to a 9 cm - petri dish (two per isolate) with Oatmeal Agar medium (OA) supplemented with chloramphenicol and streptomycin at 100 µg/ml. The fungus was cultured for seven days in a grown chamber with controlled temperature of 25°C (±2°), and photoperiod of 12/12 hours (light/darkness). Thereafter, plates were scraped out using a Drigalski spatula and 5 mL of sterilized-distilled water to induce the fungal sporulation. The dishes were incubated at the same conditions. Seven days later, the spores were harvested by adding 10 ml of distilled-sterilized water amended with 0.01% Tween-20, and carefully scraped using a Drigalski spatula.

Spore suspension was filtered through two layers of cheesecloth. Spore concentration was adjusted to $1x10^5$ spore/mL using a Neubauer counting chamber.

2.3.2 Fungicide sensitivity assay.

The glass drop method standardized by the American Phytopathological Society Committee on Fungicides Tests was used to evaluate the sensitivity to the two QoIs (azoxystrobin and pyraclostrobin) and the three SDHI (benzovindiflupyr, bixafen, and fluxapyroxad) (Dhingra & Sinclair, 1995). For EC_{50} estimation, 30 µl drop of conidial suspension were transferred on a glass adding a 30 µl fungicide solution corresponding to 0, 0.001, 0.01, 0.1, 1, 10 µg/mL fungicide final concentrations determined according to previous work (Kim et al. 2003). Salicylhydroxamic acid (SHAM) 0.5mM was added to suppress the alternative oxidase pathway. The fungicide dilutions were performed from the commercial products Amistar WG - Syngenta (azoxystrobin 500 g/L), Comet - Basf (pyraclostrobina 250 g/L), Lonselor 30 SC - Basf (fluxapyroxade 300 g/L). Bixafen and Benzovindiflupyr were diluted from their pure active ingredients. Three replicates for each concentration will be done for each isolate. The glass was placed in a plastic box with a moistened paper towel to maintain the humidity around 90% and incubated in darkness at 25°C degrees for 18-24 hours. Then, the number of germinated conidia (germ tube larger than the largest diameter of the conidium) was counted using the light microscope. The conidial germination percentage was estimated for each fungicide concentration. The EC_{50} for each of the 13 isolates was defined as the effective concentration leading to a 50% inhibition of conidia germination. A discriminatory dose for each

fungicide was defined as a median EC_{50} value and applied to all isolates in the working collection using the same glass drop method with a single dose.

2.4 *In vivo* control efficacy assays

2.4.1 Plant growth conditions.

Wheat (cv. BRS 264, classified as blast-susceptible) was sown in 2-L plastic pots filled with substrate (Tropstrato - Vida Verde), which was a mixture of pine bark, peat, and expanded vermiculite. Fertilization was performed using monoammonium phosphate (12% N and 50% P2O5) (Comissão Brasileira de Pesquisa de Trigo e Triticale, 2020). The number of plants per pot was reduced to five. Plants were kept in the greenhouse under controlled environmental conditions (±11 hour of light and 25°C ±4°C) and watered daily until inoculation time. Side-dressing fertilization were conducted weekly adding to each pot 30ml of nutritive solution prepared with 6.4mg/L KCl, 3.48mg/L $K2SO_4$, 5.01mg/L $MgSO_4.7H_2O$, 2.03mg/L $(NH_2)_2CO$, 0.009mg/L $NH_4.MO_7O_{24}.4H_2O$, 0.054mg/L H_3BO_3, 0.222mg/L $ZnSO_4.7H_2O$, 0.058mg/L $CuSO_4.5H_2O$, 0.137mg/L $MnCl_2.4H_2O$, 0.27g/L $FeSO_4.7H_2O$ and 0.37g/L disodium-EDTA prepared with distilled water (Xavier Filha *et al.*, 2011).

2.4.2 Fungicide spray and host inoculation

Wheat heads in the pre-flowering - growth stage 60 (Zadoks *et al.*, 1974) were sprayed with the fungicides at commercial doses (Fig. 3A). Three fungicides groups were used in this experiment: DMI (tebuconazole and epoxiconazole), QoI (azoxystrobin and pyraclostrobin) and SDHI (bixafen, fluxapyroxad and benzovindiflupyr). The applications were made with a manual sprayer in a volume of

800 µl of spray solution per pot (160 µl per plant). Twenty-four hours after the fungicide treatment, heads were inoculated spraying 20ml of spore suspension per pot (1 x 10^5 conidia per mL supplemented with Tween-20 1% and 0.8% gelatin) (Cruz *et al.*, 2011b, 2016a) (Fig. 3B). The spore production procedure was described before in page 24. Two PoT isolates per fungicide treatment were used in the inoculation process. The choice of the isolates was based on their performance in the *in vitro* sensitivity test. For TEBU and EPOX, those that presented the highest (Resistant, R) and lowest (Sensitive, S) values of EC_{50} were used. For QoI (AZOX and PYRA) and SDHI (BIXA, FLUX and BENZ), the isolates used in this test showed the highest and lowest germination percentage in the *in vitro* sensitivity test using a discriminatory dose. For each fungicide treatment the isolates were inoculated individually and in a mixture: Resistant (R), Sensitive (S) and Resistant+Sensitive (R+S). The subcollection of isolates used in the *in vivo* efficacy study is shown in Table 1.

Table 1. Phenotypic classification (R = resistant, S = sensitive) of nine *Pyricularia oryzae Triticum* lineage isolates with regards to fungicide resistance which were selected for inoculation in the *in vivo* control efficacy assays.Below the name of each fungicide, the recommended field dose for each active ingredient is specified.

	Fungicide[1] (mL/ha)						
Isolate code	Azox (80)	Pyra (150)	Benz (30)	Flux (50)	Bixa (65)	Epox (94)	Tebu (150)
UFVPY70	R	R	R				
UFVPY239		S					
UFVPY213			S	S		R	
UFVPY889				R			
UFVPY733					R		
UFVPY375					S		
UFVPY276						S	
UFVPY311							R
UFVPY652							S

[1]Azox= azoxystrobin, Pyra= pyraclostrobin, Benz= benzovindiflupyr, Flux= fluxapyroxad, Bixa= bixafen, Epox= epoxiconazole, Tebu= tebuconazole. The doses are those recommended for application in the field.

Following inoculation, the plants were incubated in a dark chamber for 20 hours, with 90% humidity and temperature at 27°C (Castroagudín *et al.*, 2016). The plants were moved to a growth chamber with controlled temperature at 28°C (±2°), humidity >80%, and 12/12 hours of light/darkness during seven days, until performing the disease assessments (Cruz *et al.*, 2016a). The blast disease severity (%) was visually estimated in five heads per pot using as a reference the standard area diagram developed by (Maciel *et al.*, 2013) (Fig.3C).

To determine the spore production in each treatment, the number of spores produced per milligram of rachis (spores mg/rachis) was calculated. The rachis of five heads were separated from their spikelets and surface-disinfested with sodium hypochlorite 1.25 % for 1 minute, followed by two rinses with distilled and sterilized water. The clean rachises were placed into a humidity chamber in a petri dish filled with moistened paper (9 cm of diameter). Dishes were moved to a 28°C (±2°) growing chamber with 12/12 hours of photoperiod (fluorescent light/darkness) during 72 hours to favor fungus sporulation (Pizolotto *et al.*, 2019; Kovaleski *et al.*, 2020) (Fig. 3D). After this period, the five rachis were placed together into a 15 mL Falcon tube filled with 5 mL of autoclaved water amended with Tween 20 (2 drops per liter) and shaken for 10 seconds using a Vortex Mixer (KASVI, K45-2810) (Kovaleski et al. 2020) (Fig. 3E). The number of conidia in the suspension was determined using a hemocytometer chamber (Fig. 3F). After the spore count, the rachis were dried for 3 days at 60°C and weighed on an analytical balance to obtain the dry weight (Fig. 3G). All *in vivo* experiments were conducted twice.

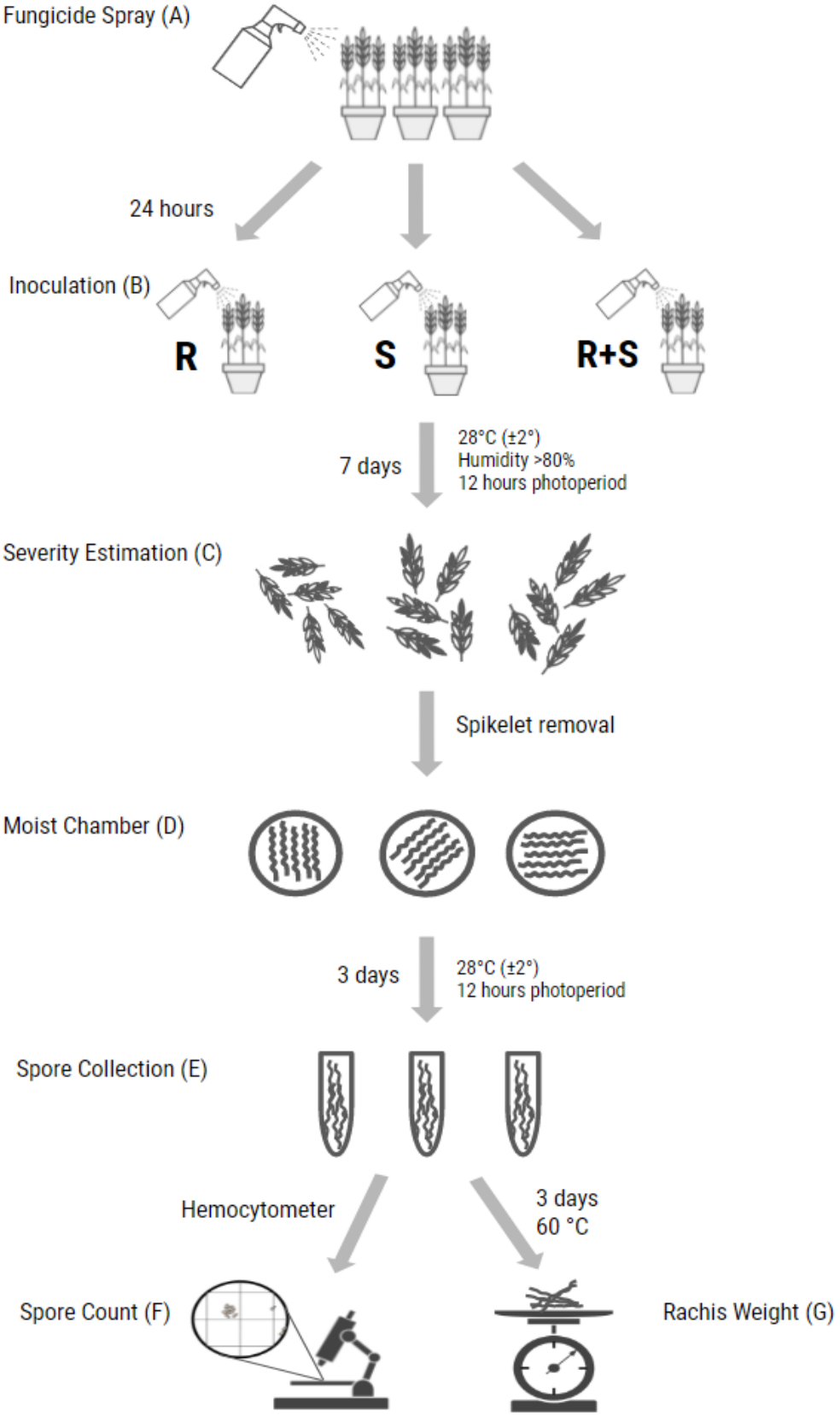

Figure 3. Diagram illustrating the steps of the *in vivo* assays aimed at evaluating the effect of the *Pyricularia oryzae Triticum* pathotype (PoT) inoculum composition (R = resistant, S = sensitive, or a mix of both) and fungicides for the control of wheat blast severity and sporulation.

2.5 Data analysis

For the *in vitro* assays, the *drc* package (Ritz *et al.*, 2015) was used to fit and select the best nonlinear model (based on the lowest Akaike's information criteria) for the response in mycelial growth (DMI) or germination percentage (QoI and SDHI) to the exposure of increasing fungicide concentrations. The data from two runs of the experiments per fungicide were combined for analysis. The EC_{50} for each combination of isolate and fungicide was obtained using the *ec50estimator* R package (Alves, 2020).

In the discriminatory dose test for SDHI and QoI the isolates were considered sensitive (S) to a specific fungicide when the number of germinated conidia was less than 50%, and were considered resistant (R) when equaling or exceeding 50% germination (Ishii *et al.*, 2009).

To compare the PoT and the non-PoT combined in terms of EC_{50} (DMIs) and germination percent (SDHIs and QoIs) a nonparametric statistical bootstrapping method was used. This consists of randomly sampling a data set with replacement, then performing the analysis individually on each bootstrapped replicate. If the difference between the parameters of interest is different from zero (confidence interval not including zero) the means of the two groups were deemed statistically different. The *two.boot* function of the “simpleboot” R package was used (Peng, 2019). For assessment of cross-resistance for DMI fungicides, EC_{50} values were subjected to Spearman’s rank correlation analysis ($P = 0.05$). The data from two runs of the experiment were combined for each fungicide.

For the *in vivo* control efficacy assays data, a general linear model was fitted to data on disease severity and the log of the spores/mg rachis. The best family for the data distribution was selected based on simulation of the residuals provided by

DHARMa R package (Hartig, 2021), which provides *p-values* for normality of residuals, overdispersion and outliers. Three factors were defined: treatment (treated, untreated), the experiment number (exp-1, exp-2) and the inoculum type (R, S, R+S). The significance of the three-way and two-way interactions were analyzed and, if not significant, the single factor was analyzed. Estimated means were submitted to analysis of variance using the *lm()* function of the base R package. Means were compared by Tukey's test at 5% probability ($P < 0.05$) using both *emmeans* R package (Lenth *et al.*, 2020) and *multcomp* (Hothorn *et al.*, 2008). For the spore production data, the means of the log(spores/mg rachis) were back-transformed to the original values using the *back.emmeans* function. Data analysis was performed in R (R Core Team, 2021).

2.6 Genotypic Characterization

All PoT isolates (n = 9) used in the *in vivo* assay were genotypically characterized for the resistance to each of the three fungicide groups (Table 1). The *cytB* region was amplified for two extra isolates with different responses to QoI fungicides (isolates 309 and 376, S and R to PYRA respectively; both resistant to AZOX). A subcollection of 12 strains of non-PoT isolates was included in the molecular screening. The selection was based on their performance in the sensitivity *in vitro* test. For the DMI fungicide, two high EC_{50} isolates and two low EC_{50} isolates were selected. For QoI and SDHI, the selection was based on the germination (%) values obtained in the discriminatory dose test. Two high germination (%) isolates and two low germination (%) isolates were selected for both groups of fungicides. For SDHI, an additional isolate with variable response between the three individual fungicides was included. The non-PoT isolates are listed in Table 2.

Table 2. Phenotypic classification (R = resistant, S = sensitive) of twelve non-Pot isolates with regards to fungicide resistance which were selected for sequencing.

Isolate code	QoI		SDHI			DMI	
	Azox	Pyra	Benz	Flux	Bixa	Epox	Tebu
UFVPY92	R	R					
UFVPY463	R	R	R	R	S		
UFVPY209	S	S					
UFVPY21	S	S					
UFVPY158			R	S	S		
UFVPY656			R	S	S		
UFVPY92			S	R	S		
UFVPY120			S	S	S		
UFVPY112						R	R
UFVPY108						R	R
UFVPY1						S	S
UFVPY200						S	S

Azox= azoxystrobin, Pyra= pyraclostrobin, Benz= benzovindiflupyr, Flux= fluxapyroxad, Bixa= bixafen, Epox= epoxiconazole, Tebu= tebuconazole.

2.6.1 Growth of *Pyricularia* spp. isolates and DNA extraction

A single filter paper of each isolate was put to grow on a potato-dextrose-agar (PDA) dish and incubated at 25±5°C under photoperiod 12/12 h (fluorescent light/darkness). A 6 mm-mycelial block from a 5 days-old colony was transferred to a 50 mL falcon tube filled with 20 mL of liquid-medium (6 g casamino-acids, 6 g yeast extract, and 10 g sucrose per 1 liter). The tubes were shaken for 7 days at 150 rpm under room temperature (23-26°C) and ambient light. The mycelium was recovered through two layers of cheesecloth and let to dry at sterely-ambient temperature for three hours, and freeze-dried within a 2 mL microtubes for 24 hours (Farman *et al.*, 2017; Urashima *et al.*, 2017) using a CoolSafe Freeze Dryers (SCANVAC). The mycelium ball was manually crushed into the microtube wall until it formed a powder, which was resuspended with 1 mL lysis buffer (100 mM Tris-HCl, pH8; 0.5 M NaCl, 10 mM EDTA; 1 % SDS) and heated at 65°C for 30 minutes. Adding 700 µl

phenol:chloroform:isoamyl-alcohol (25:24:1) and heated at 65°C for 30 minutes. Subsequently, centrifuged at 14,000 rpm for 15 minutes, and carefully transferred 0.8 µl of aqueous phase to a new identified microtube, where was added 450µl of cool isopropanol and centrifuged at 14,000 rpm for 10 minutes to form the pellet of DNA. Supernatant was carefully discarded. The DNA was washed by 1 mL of 70% pure ethanol by centrifuging for 5 minutes at 14,000 rpm. Newly formed DNA-pellet was dried at room temperature for 60 minutes. The DNA was resuspended with 100 µl TE + 2 µl RNAse A (1 µg/ml) and stored at 4°C overnight before being placed in the -20°C freezer (Farman *et al.*, 2017). The DNA concentration was estimated using a spectrophotometer NanoDrop 2000 (Thermo Scientific™). DNA-concentration was adjusted to 100ng/µl by adding TE.

2.6.2 PCR amplification of a *cyp51A* gene

The PCR assays were performed with 1µl of genomic DNA (100ng/µl) using the GoTaq® Colorless Master Mix, according to the manufacturer's specifications (Promega). Five PCR primers in different combinations were used to amplify the CYP51A gene: CYP51A_-278F + CYP51A_757R; CYP51A_-278F + CYP51A_1345R and CYP51A_662F + CYP51A_1749R (Poloni *et al.*, 2021) (Table 3). Reactions were carried out at a thermal cycler MyGene™ (Model MG96G) using the following cycling conditions: initial denaturation at 95°C for 5 minutes, followed by 35 cycles at 95°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute; and final extension at 72°C for 5 minutes. Three DNA fragments to cover the total *cyp51A* gene were amplified and sequenced.

2.6.3 PCR amplification of *sdh* gene subunits

Primers for the amplification of *sdhB, sdhC* and *sdhD* genes encoding the SDH subunits that form the SDHI binding site were designed using the *online* software Primer3web version 4.1.0 (Kõressaar *et al.*, 2018). For this purpose, sequences of these genes already published were used. The properties and quality of primers were analyzed by the *online* OligoAnalyzer tool (Integrated DNA Technologies OligoAnalyzer - RRID:SCR_001363). The PCR assays were performed with 1µl of genomic DNA (100ng/µl) using the GoTaq® Colorless Master Mix, according to the manufacturer's specifications (Promega). To amplify the different subunits, the following pairs of primers were used: SDHB_F3 + SDHB_R2 (*sdhB*); SDHC_F1 + SDHC_R1 (*sdhC*) and SDHD_F1 + SDHD_R1 (*sdhD*) (Table 3). Reactions were carried out at a thermal cycler MyGene™ (Model MG96G) using the following cycling conditions: initial denaturation at 95°C for 5 minutes, followed by 35 cycles at 95°C for 45 seconds, 56°C for 45 seconds and 72°C for 1 minute; and final extension at 72°C for 8 minutes. Three DNA fragments corresponding to each subunit were amplified and sequenced.

2.6.4 PCR amplification of a *cytB* gene

The PCR assays were performed with 1µl of genomic DNA (100ng/µl) using the GoTaq® Colorless Master Mix, according to the manufacturer's specifications (Promega). The PCR primers (PgCytb-F1 + PgCytb-R1) used to amplify the *cytB* gene were those used by Castroagudín et al. (2015) (Table 3). Reactions were carried out at a thermal cycler MyGene™ (Model MG96G) using the following cycling conditions: 95°C for 10 minutes, followed by 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 2 minutes, and a final extension of 5 minutes at 72°C. The DNA fragment obtained was amplified and sequenced.

Table 3. Specific PCR primers used to amplify *cyp51A*, *sdhB, sdhC, sdhD* and *cytB* genes.

Gene	Primers combination	Sequence (5´ - 3´)	Product (bp)	Reference
cyp51A	CYP51A_-278F	CTTTTGTCACTTGTTCTCTGCC	879	Poloni et al. (2020)
	CYP51A_757R	TGAGGTCCATGTAAACATCG		
	CYP51A_-278F	CTTTTGTCACTTGTTCTCTGCC	1470	
	CYP51A_1345R	CAAAGGGCAGGTAAGGACTC		
	CYP51A_662F	GCCCCATCAACTTCCTAG	1085	
	CYP51A_1749R	AGAGATATGCCTCATTGCTAAA		
sdhB	SDHB_F3	CGCCTCTCGGTACTTACGTC	1052	This study
	SDHB_R2	CGAAAGCCATCTCCTTCTTG		
sdhC	SDHC_F1	CACAAGCACGGTATGTGTAACC	911	
	SDHC_R1	ACTCTGCACATTGTGGTAAACG		
sdhD	SDHD_F1	AGCAAGTAAACTCGGCAAAGAC	882	
	SDHD_R1	TCCTCTAcGACCCTCTCTTTCTT		
cytB	PgcytbF1	AGTCCTAGTGTAATGGAAGC	510	Castroagudín et al. (2015)
	PgcytbR1	ATCTTCAACGTGTTTAGCACC		

2.6.5 Sequencing and bioinformatic analysis

The PCR products corresponding to each amplificated gene for each isolate were sent to ACTGene (Rio Grande do Sul, Brazil). They were purified using ExoSAP-ITTM kit and then the DNA sequencing was performed on AB 3500 Genetic Analyzer. To obtain the consensus sequences was used BioEdit (RRID:SCR_007361) software (Hall, 1999). The identification of the mutation points and its effects on the amino acid sequences were analyzed using Geneious prime® software version 2022.0.1 (Kearse *et al.*, 2012). The mutations G143A, F129L and G137R, which confer QoI resistance, were manually searched in the amplified partial sequence of *cytB* genes. For *cyp51A* gene sequences and the gene sequences to codify the different subunits of the SDH (*sdhB, sdhC* and *sdhD*), associations

between mutations and sensitivity to DMI and SDHI fungicides was respectively searched.

3 RESULTS

3.1 Sensitivity to DMI fungicides

Both DMIs (TEBU and EPOX) reduced the mycelial growth more effectively with the increase of the active ingredient concentration for all tested isolates. In general, the isolates were slightly more sensitive to EPOX than to TEBU: the EC_{50} values ranged from 0.152 to 1.577 µg/mL (mean = 0.926, median = 1.016) for TEBU and from 0.003 to 1.188 µg/mL (mean = 0.546, median = 0.572) for EPOX. When the lineages grouped as PoT or non-POT were compared, the mean difference in the EC_{50} suggested a difference between the lineages for both fungicides (Fig. 4 A, B). When the isolates were exposed to TEBU, the mean EC_{50} was 1.077 and 0.668 µg/mL for PoT and non-PoT respectively. When exposed to EPOX, the mean EC_{50} was 0.644 and 0.379 µg/mL, respectively. The correlation analysis showed a significant and positive association between the EC_{50} values obtained for EPOX and TEBU (ρ = 0.81, $P < 0.01$), which was suggestive of cross-resistance (Fig. 4 C).

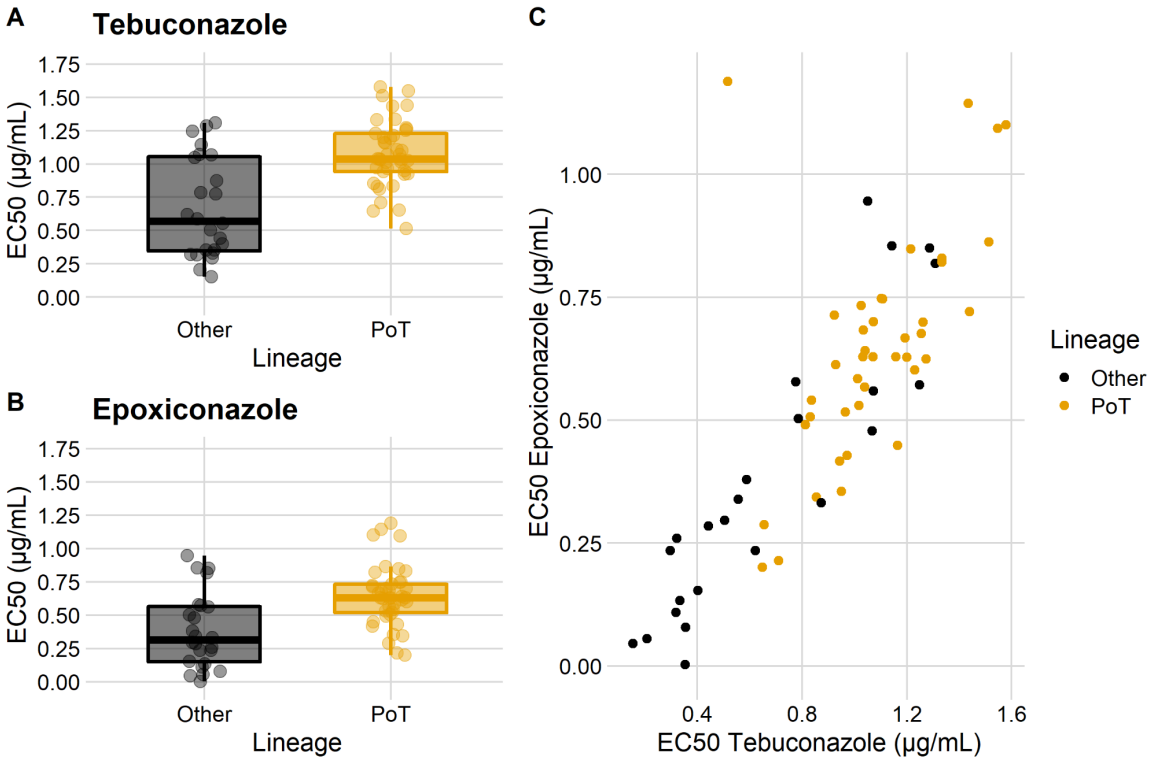

Figure 4. Box-plot for the distribution of EC_{50} for DMI fungicides tebuconazole (A) and epoxiconazole (B). Scatter-plot for the relation between the EC_{50} of both fungicides (C). Different colors represent the different lineages. Each dot represents a single isolate. The solid line in the box represents the median. The lower and upper hinges correspond to the interquartile (between 25 and 75%) range (IQR). The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 IQR from the hinge.

3.2 Sensitivity to QoI fungicides

3.2.1 EC_{50} and discriminatory doses

Firstly, the EC_{50} values were determined for 13 isolates for which the germination was inhibited differently depending on the QoI. AZOX did not inhibit the conidial germination even at an extra highest concentration (100 µg/mL) included in the second run of the experiment (Fig. 5A, C). On the other hand, the EC_{50} values for PYRA ranged from 0.011 to 3.613 µg/mL (mean = 1.579). Based on the distribution and median EC_{50} value, the discriminatory dose for PYRA was defined as 1.33 µg/ml. Due to the high EC_{50} values obtained for AZOX via fitting of the models to the

germination data (min = 20,359, max = 4.11 x 10^{79} and mean = 6.31 x 10^{78}), the discriminatory dose of 5 µg/ml was defined based on previous works about the sensitivity of *P. oryzae* isolates to AZOX (Castroagudin et al. 2015).

3.2.2 Screening using discriminatory doses

A wide range of spore germination percentage on the discriminatory dose was found for the larger sample of 62 isolates. For PYRA, the percentage values ranged from 0.68 to 96.24 % (mean = 34.38%) and for AZOX they ranged from 1.56 to 100 % (mean = 78.19%). The proportion of isolates with germination values above 50% were 21/62 for PYRA and 48/62 for AZOX (Fig. 5B, D). There was a marked difference in germination between the PoT and non-PoT lineages. For AZOX, while all PoT germinated close to 100% for all isolates, for a few non-PoT isolates, the germination was almost completely inhibited, but more than half of the isolates in this group germinated more than 50% (Fig. 5C). For PYRA, germination of non-PoT was lower than 25%, while a wide range was observed for PoT (Fig. 5D)

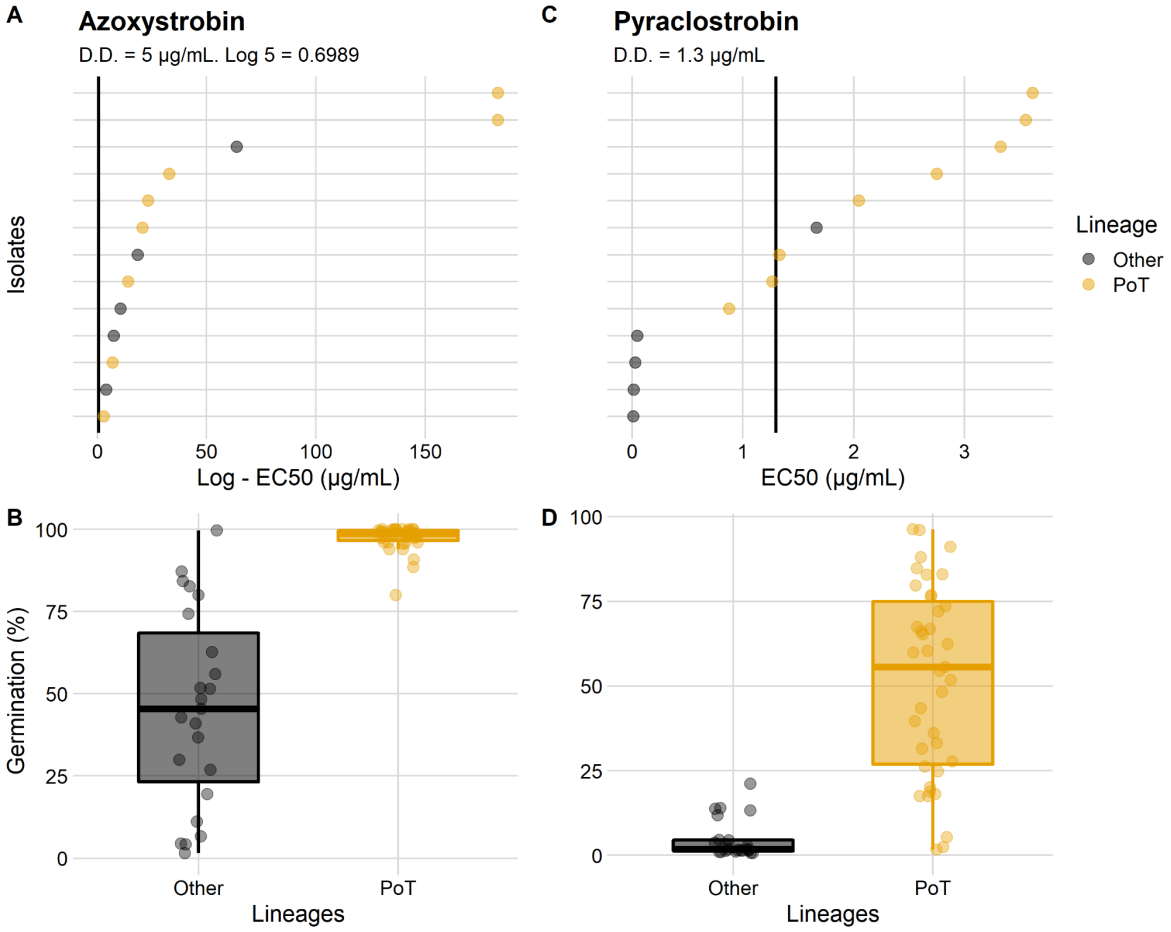

Figure 5. Distribution of EC_{50} values for the sub-collection of thirteen isolates used to obtain the discriminatory dose for the QoI fungicides azoxystrobin (A) and pyraclostrobin (C). Box-plot for the distribution of germination (%) values for the germination test using the discriminatory dose for azoxystrobin (B) and pyraclostrobin (D). The different colors represent the different lineages. Each dot represents a single isolate. The dark-vertical line in A and C represents the location point of the discriminatory dose. The solid line in the box represents the median. The lower and upper hinges correspond to the interquartile (between 25 and 75%) range (IQR). The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 IQR from the hinge.

3.3 Sensitivity to SDHI fungicides.

3.3.1 EC_{50} and discriminatory doses

Conidial germination generally decreased in the subcollection of 13 isolates, in response to the increase of the SDHI concentration, regardless of the active ingredient. The levels of sensitivity contrasted sharply among the three SDHIs based

on the EC_{50} with FLUX > BIXA > BENZ. For FLUX, EC_{50} ranged from 3.260 to 136.00 µg/mL (mean = 36.440 µg/mL); for BIXA they ranged from 1.004 to 52.966 µg/mL (mean = 9.422 µg/mL); and for BENZ they ranged from 0.001 and 0.044 µg/mL (mean = 0.009 µg/mL) (Fig. 6A, C, E). Based on the distribution and median EC_{50} value for each fungicide, the following discriminatory doses were defined: FLUX = 13.400, BIXA = 4.115, and BENZ = 0.005 µg/mL.

3.3.2 Screening using discriminatory doses

Similar to the QoIs, a wide range of germination levels was detected in the collection of 62 tested isolates submitted to the three SDHIs at their respective discriminatory doses. The germination percent values ranged from 0.67 to 68.5 % for BIX (mean = 13.67), 0.33 to 98.9% for FLUX (mean = 50.99); and 0.34 and 100 % for BENZ (mean = 62.65). The number of isolates with germination percentage above the 50% threshold varied across the fungicides. Only five isolates for BIX, 33 isolates for FLUX and 40 isolates for BENZ. When isolates belonging to PoT or non-PoT were compared, they showed statistical differences based on the bootstrap. The PoT isolates exhibited the highest mean percent germination values of 19.42, 67.48 and 78.62 % for BIX, FLUX and BENZ respectively. On the other hand, percent mean germination values for non-PoT were 3.92, 23.05 and 35.58 % for BIXA, FLUX and BENZ respectively (Fig. 6B, D, F).

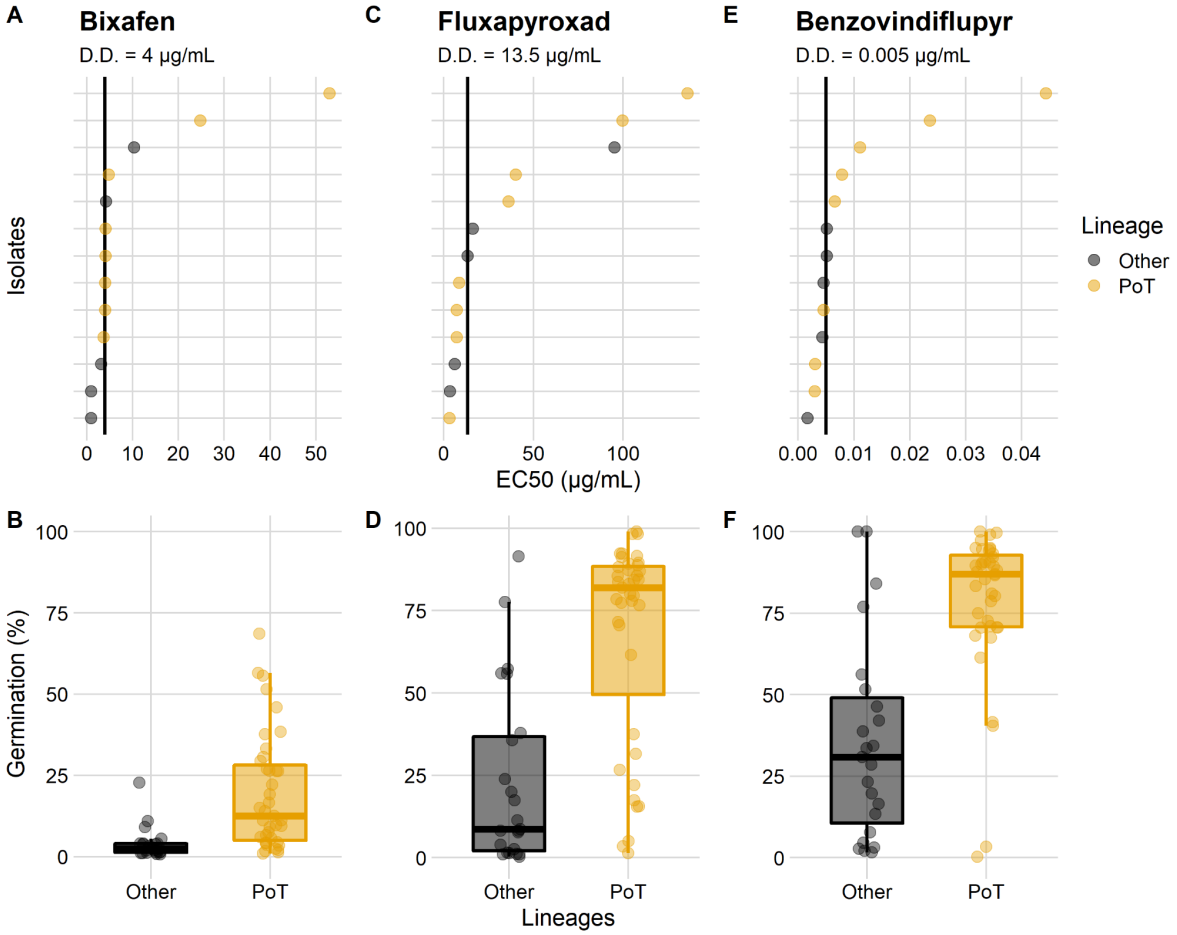

Figure 6. Distribution of EC_{50} values for the sub-collection of thirteen isolates used to obtain the discriminatory dose for the SDHI fungicides bixafen (A), fluxapyroxad (C) and benzovindiflupyr (E). Box-plot for the distribution of germination (%) values for the germination test using the discriminatory dose for bixafen (B) and fluxapyroxad (D) and benzovindiflupyr (F). in all-collection isolates. Different colors represent the different lineages. Each dot represents a single isolate. The dark-vertical line in A, C and E represents the location point of the discriminatory dose. The solid line in the box represents the median. The lower and upper hinges correspond to the interquartile (between 25 and 75%) range (IQR). The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 IQR from the hinge.

3.4 *In vivo* control efficacy assays

3.4.1 DMI fungicides

Both disease severity and spore production were significantly reduced for treatments sprayed with either TEBU or EPOX according to the ANOVA test ($P < 0.0001$). For TEBU, the triple interaction (treatment-inoculum-experiment) was not

significant (*P = 0.58*). Neither was the inoculum nor the experiment as single effects (*P= 0.83* and *P= 0.29* respectively). Control efficacy of disease severity was greater than 88%. Similar pattern was observed for spore production (Fig 7A, B, Table 4).

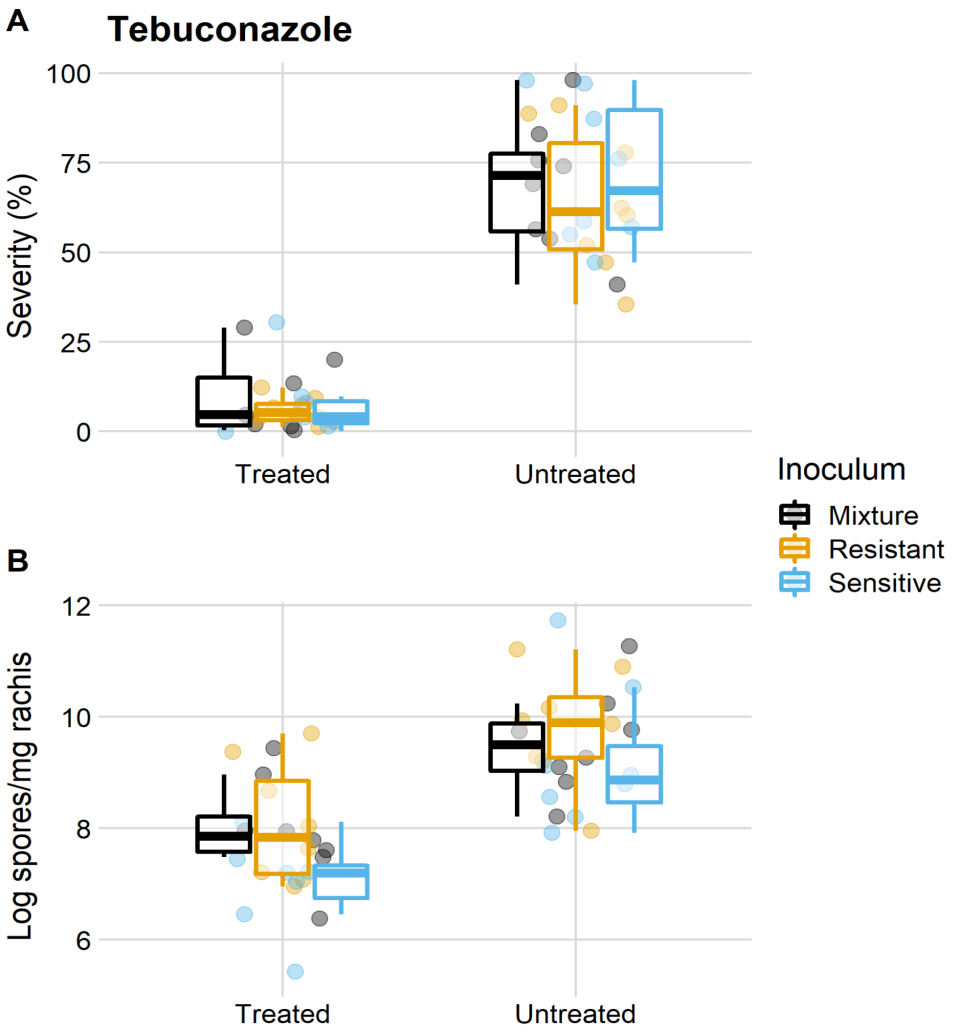

Figure 7. Box-plot for blast severity (%) (A) and logarithm of spores/mg rachis (B) for wheat heads treated and untreated with tebuconazole. Different box-plot colors represent the condition of the isolate inoculated. Each dot represents a single isolate. The solid line in the box represents the median. The lower and upper hinges correspond to the interquartile (between 25 and 75%) range (IQR). The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 IQR from the hinge.

For EPOX, only one interaction factor (treatment x inoculum) was significant (*P= 0.02*) for the severity variable. The greatest control efficacy (83.9%) was

observed on heads inoculated with a susceptible isolate. When heads were inoculated with the resistant isolate and a mixture of isolates, control efficacy was lower than 60% (Fig. 8A). There was an effect of the experiment (*P= 0.0006*) on the production of spores with greater reduction in the spores/mg rachis in the experiment 1 (> 80%), while the reduction was ten times lower in the second experiment run (Fig. 8B, Table 4).

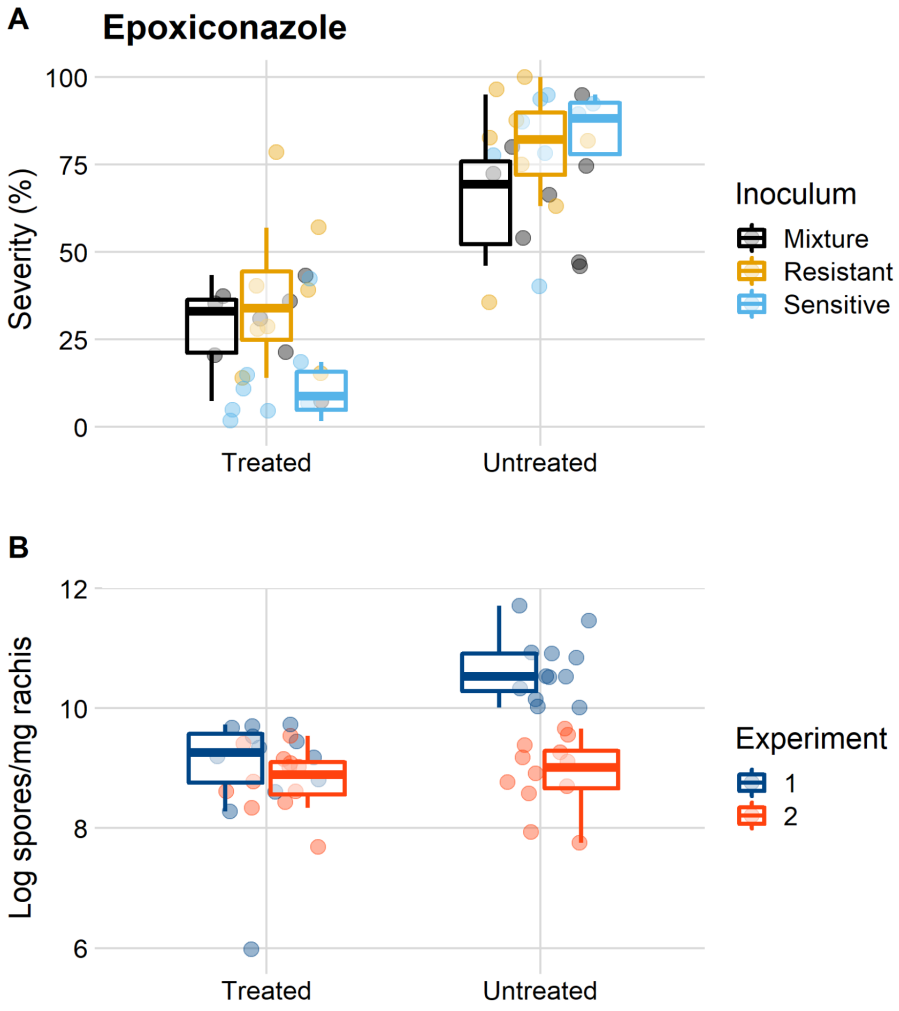

Figure 8. Box-plots for blast severity (%) (A) and logarithm of spores/mg rachis (B) estimated on wheat heads treated and untreated with epoxiconazole. Different box-plot colours in A represent the condition of the isolates inoculated, and different box-plot colours in B represent the experiment number. Each dot represents a single isolate. The solid line in the box represents the median. The lower and upper hinges correspond to the interquartile (between 25 and 75%) range (IQR). The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 IQR from the hinge.

Table 4. General linear model estimates for the means of wheat blast severity and number of spores/mg rachis as a result of the *in vivo* control efficacy assay for DMI fungicides in heads inoculated with resistant (R), sensitive (S) and a mixture of isolates (S+R) in two experiments (Exp-1, Exp-2).

Fungicide	Response	Factor - Level	Untreated	Treated	Control (%)
Tebuconazole	Severity (%)	-	68.4 a	7.6 b	88.9
	Spores/mg rachis	-	13,770 a	1,557 b	88.6
Epoxiconazole	Severity (%)	Isolate - S	81.7 Aa	13.1 Ab	83.9
		Isolate - R	77.8 Aa	37.6 ABb	51.7
		Isolate - S+R	66.9 Aa	29.1 ABb	56.5
	Spores/mg rachis	Exp - 1	42,760 Aa	7,758 Ab	81.8
		Exp - 2	7,341 Ba	6,690 Aa	8.5

Means with different uppercase letters in the column and lowercase in the row differ from each other at 5% probability.

3.4.2 Qol fungicides

According to the Anova test, the treatment with AZOX did not significantly reduce disease severity (*P = 0.35*) and spore production (*P= 0.08*) compared with the untreated plants. However, these variables were affected by experimental run (*P= 0.0004* and *P= 0.009* respectively). Control efficacy for disease severity in the second run was 19%, more than five times higher than the control efficacy in the first run of the experiment (Fig. 9A, Table 5). For spore production, control efficacy was more than two times higher in the first compared with the second run of the experiment (Fig. 9B, Table 5).

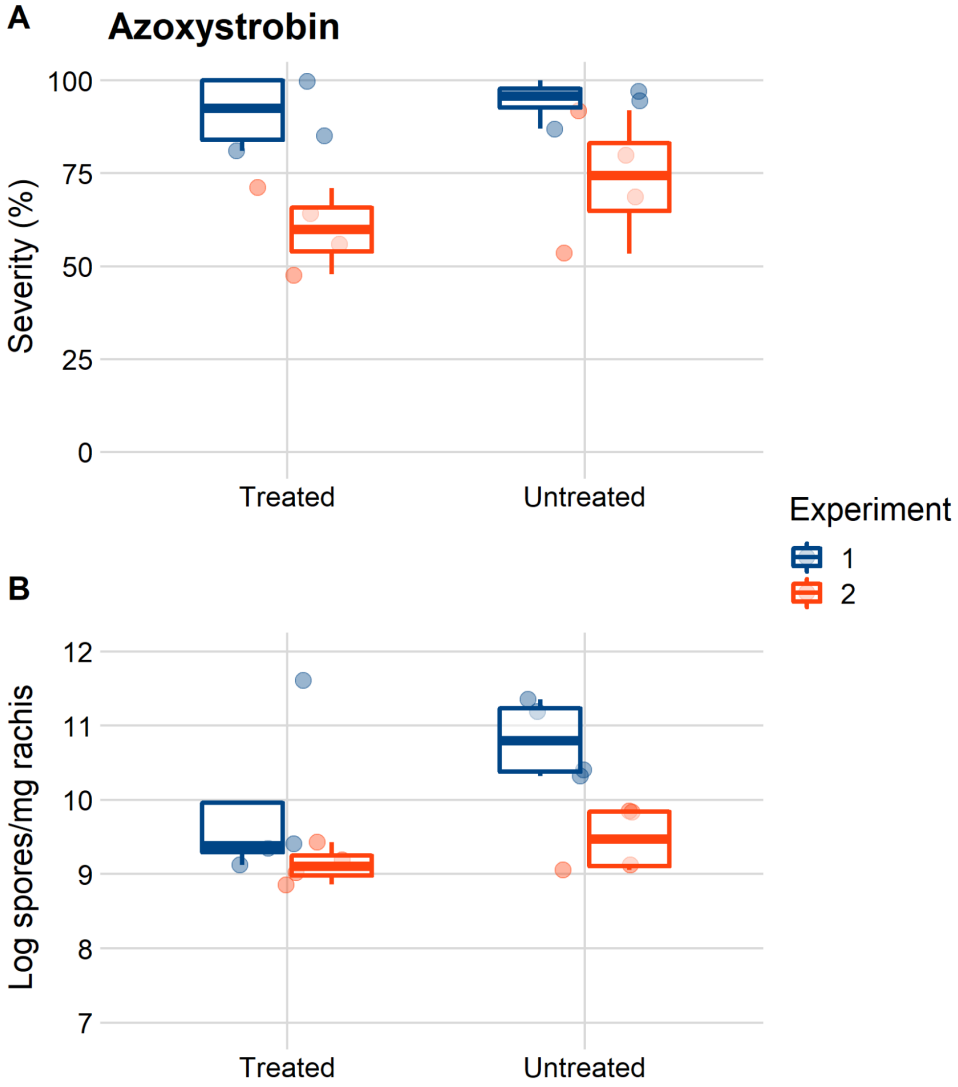

Figure 9. Box-plot for blast severity (%) (A) and logarithm of spores/mg rachis (B) for wheat heads treated and untreated with azoxystrobin. Different box-plot colours represent the experiment number. Each dot represents a single isolate. The solid line in the box represents the median. The lower and upper hinges correspond to the interquartile (between 25 and 75%) range (IQR). The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 IQR from the hinge.

For PYRA, ANOVA results showed a significant reduction in disease severity on the fungicide-treated heads ($P < 0.0001$) which also depended on the inoculum ($P= 0.02$). The highest control efficacy (~84 %) was obtained when heads were inoculated with a sensitive isolate. For heads inoculated with the resistant isolate, the control efficacy was slightly lower, and the lowest values (31 %) were obtained for the

mix of isolates (Fig. 10A, Table 5). For spore production, the triple interaction (treatment-inoculum-experiment) effect was not significant (*P= 0.10*), but there was evidence of experiment effect (P = 0.038). The control efficacy in the first run was more than twice that of the second run (Fig. 10B, Table 5).

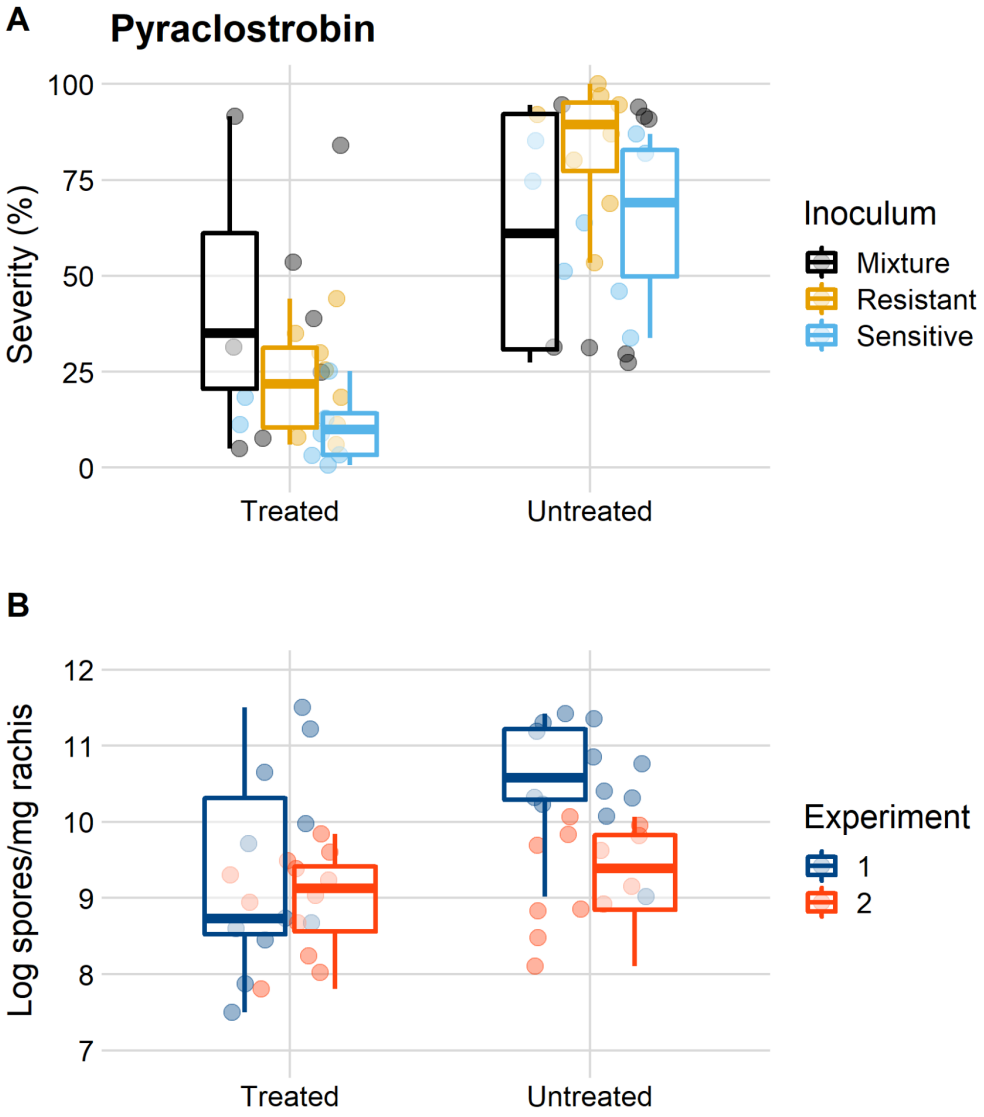

Figure 10. Box-plots for blast severity (%) (A) and Logarithm of spores/mg rachis (B) for wheat heads treated and untreated with pyraclostrobin. Different box-plot colours in A represent the condition of the isolates inoculated, and different box-plot colours in B represent the experiment number. Each dot represents a single isolate. The solid line in the box represents the median. The lower and upper hinges correspond to the interquartile (between 25 and 75%) range (IQR). The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 IQR from the hinge.

Table 5. General linear model estimates for the means of wheat blast severity and number of spores/mg rachis as a result of the *in vivo* control efficacy assay for QoI fungicides in heads inoculated with resistant (R), sensitive (S) and a mixture of isolates (S+R) in two experiments (Exp-1, Exp-2).

Fungicide	Response	Factor - Level	Untreated	Treated	Control (%)
Azoxystrobin	Severity (%)	Exp - 1	94.7 Aa	91.5 Aa	3.3
		Exp - 2	73.6 Ba	59.7 Ba	18.8
	Spores/mg rachis	Exp - 1	49,788 Ba	19,441 Aa	60.9
		Exp - 2	12,904 Aa	9,179 Aa	28.6
Pyraclostrobin	Severity (%)	Isolate - S	65.5 Aa	10.5 Ab	83.9
		Isolate - R	84.1 Aa	22.3 ABb	73.6
		Isolate - S+R	61.3 Aa	42.1 Ba	31.3
	Spores/mg rachis	Exp - 1	40,259 Aa	9,097 Ab	77.4
		Exp - 2	10,595 Ba	7,826 Aa	26.13

Means with different uppercase letters in the column and lowercase in the row differ from each other at 5% probability.

3.4.3 SDHI fungicides

For BIXA, the triple interaction (treatment-inoculum-experiment) was not significant (*P = 0.91*) for the severity. Neither were the inoculum nor the experiment number as single effects (*P= 0.60* and *P= 0.36* respectively). The fungicide treatment was the only factor that significantly influenced the reduction in severity (%) in around 48% (*P< 0.0001*) (Fig 11A, Table 6). According to the Anova test, the treatment with BIXA did not significantly reduce spore production (Fig 11B, Table 6).

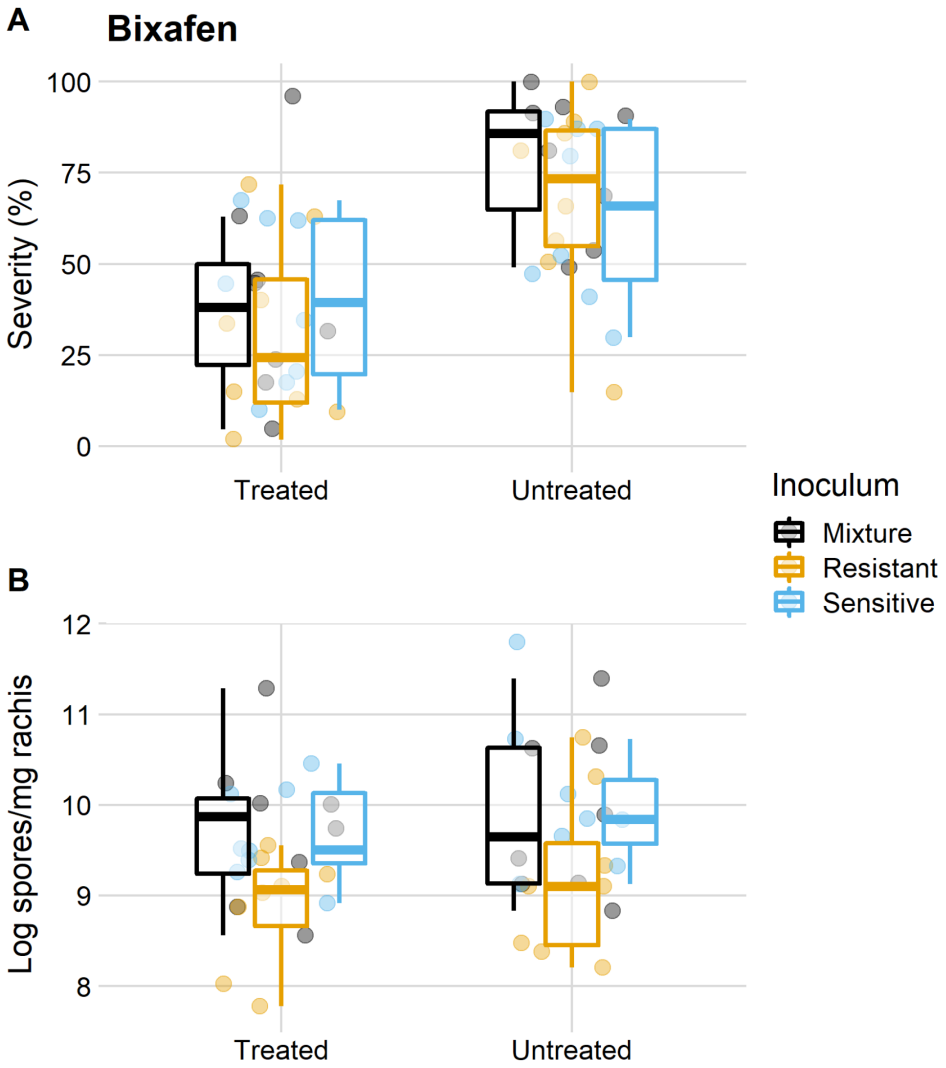

Figure 11. Box-plot for blast severity (%) (A) and logarithm of spores/mg rachis (B) for wheat heads treated and untreated with Bixafen. Different box-plot colours represent the condition of the isolate inoculated. Each dot represents a single isolate. The solid line in the box represents the median. The lower and upper hinges correspond to the interquartile (between 25 and 75%) range (IQR). The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 IQR from the hinge.

When the heads were treated with FLUX, the triple interaction was not significant according to the ANOVA test for the reduction of severity and sporulation (*P= 0.85*), but the effects of treatment and experiment alone were significant (*P*< 0.0001). For disease severity, control efficacy was higher in the first than in the second experiment (Fig 12A, Table 6). For spore production, control efficacy in the

first experiment was almost twice (75%) the value than the efficacy determined in the second experiment (Fig 12B, Table 6).

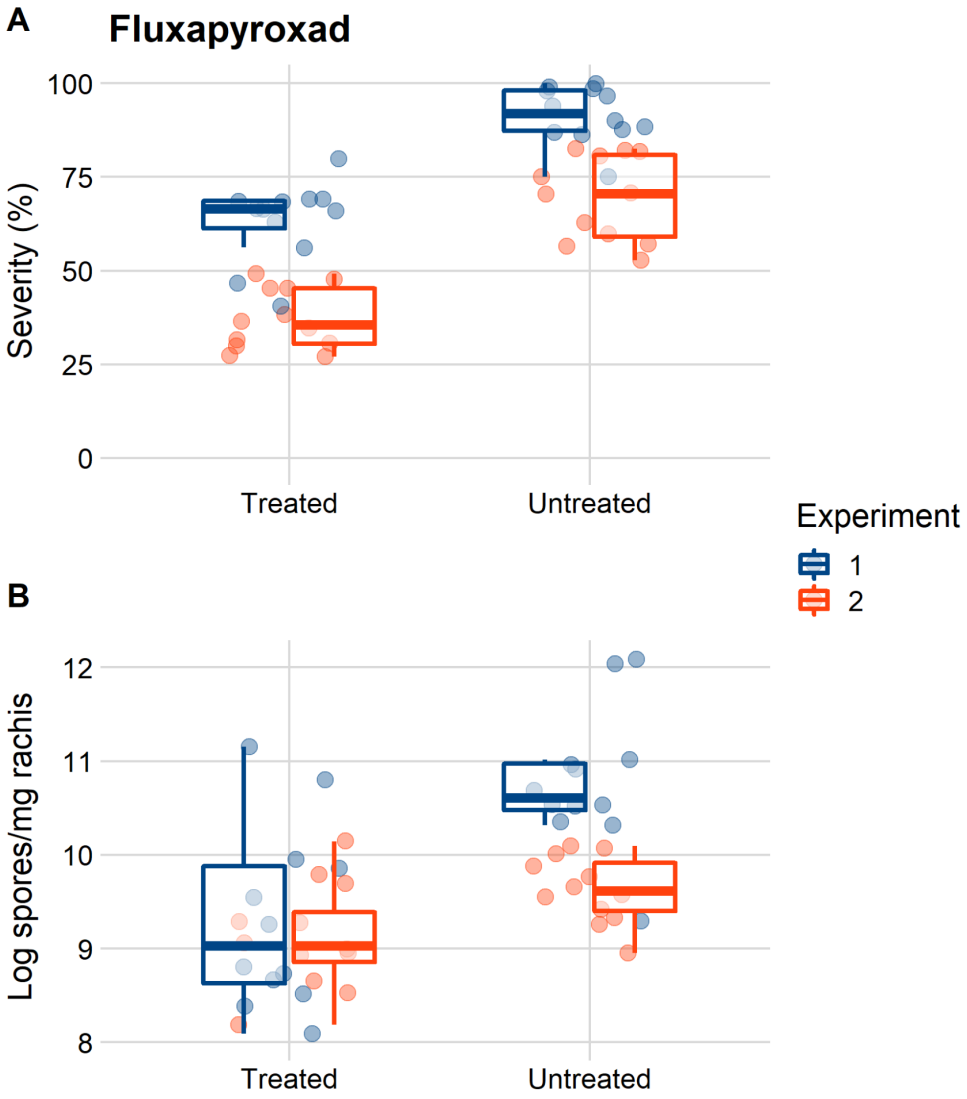

Figure 12. Box-plot for blast severity (%) (A) and logarithm of spores/mg rachis (B) for wheat heads treated and untreated with fluxapyroxad. Different box-plot colours represent the experiment number. Each dot represents a single isolate. The solid line in the box represents the median. The lower and upper hinges correspond to the interquartile (between 25 and 75%) range (IQR). The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 IQR from the hinge.

For BENZ, the treatment and experiment interaction was significant for both disease severity and spore production ($P < 0.001$). The control efficacy values for

both variables were above 94%, the highest among all fungicides (Fig 13A, B, Table 6).

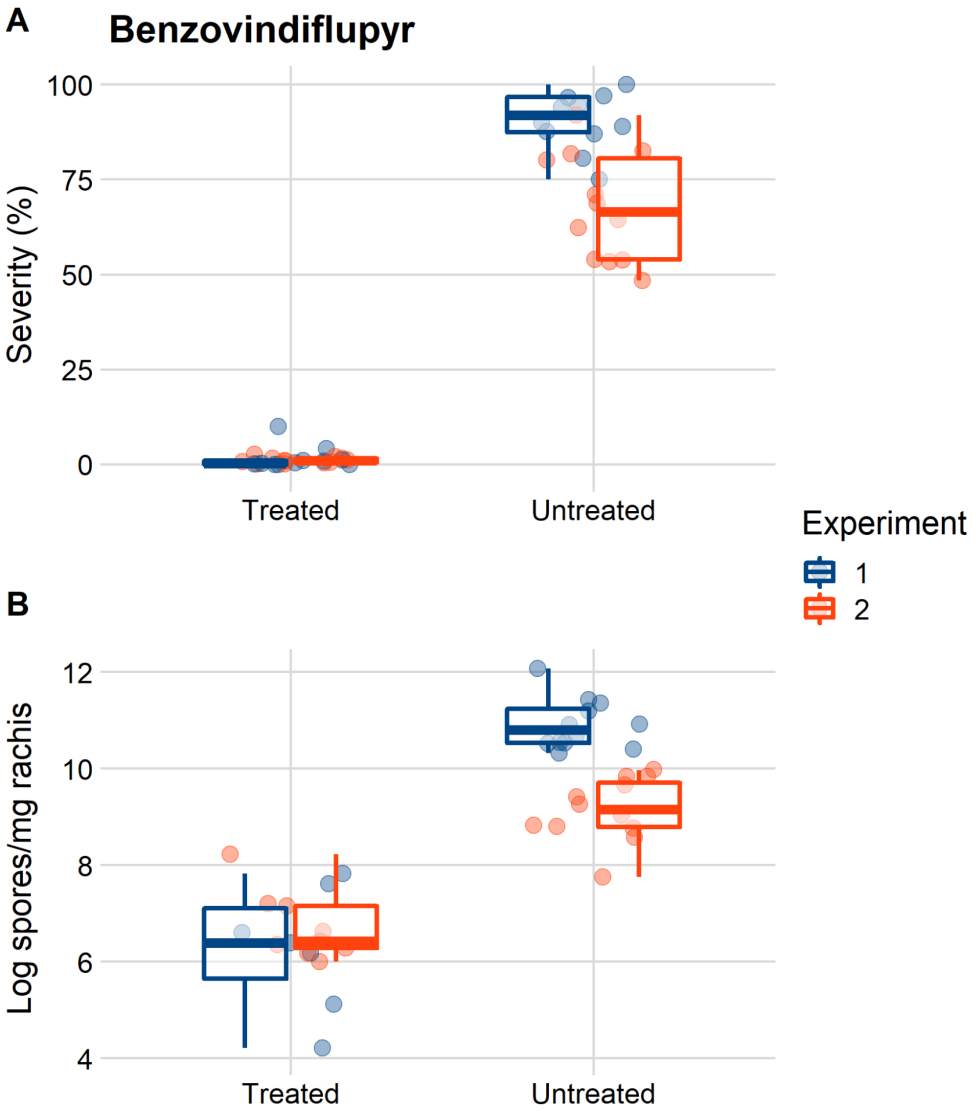

Figure 13. Box-plot for blast severity (%) (A) and logarithm of spores/mg rachis (B) for wheat heads treated and untreated with Benzovindiflupyr. Different box-plot colors represent the experiment number. Each dot represents a single isolate. The solid line in the box represents the median. The lower and upper hinges correspond to the interquartile (between 25 and 75%) range (IQR). The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 IQR from the hinge.

Table 6. General linear model estimates for the means of wheat blast severity and number of spores/mg rachis as a result of the *in vivo* control efficacy assay for SDHI fungicides in heads inoculated with resistant (R), sensitive (S) and a mixture of isolates (S+R) in two experiments (Exp-1, Exp-2).

Fungicide	Response	Factor - Level	Untreated	Treated	Control (%)
Bixafen	Severity (%)	-	72.2 a	37.2 b	48.5
	Spores/mg rachis	Isolate - S	23,264 Aa	15,748 Aa	32.3
		Isolate - R	9,950 Aa	7,164 Aa	27.9
		Isolate - S+R	19,603 Aa	17,312 Aa	11.7
Fluxapyroxad	Severity (%)	Exp - 1	91.7 Aa	63.3 Ab	30.9
		Exp - 2	69.4 Aa	36.9 Ba	46.8
	Spores/mg rachis	Exp - 1	54,340 Aa	14,006 Ab	74.2
		Exp - 2	15,675 Ba	9,875 Aa	37.0
Benzovindiflupyr	Severity (%)	Exp - 1	90.7 Aa	0.6 Ab	99.3
		Exp - 2	67.0 Ba	0.9 Ab	98.6
	Spores/mg rachis	Exp - 1	58,268 Aa	259 Ab	99.5
		Exp - 2	10,006 Ba	533 Ab	94.7

Means with different uppercase letters in the column and lowercase in the row differ from each other at 5% probability.

3.5 Genotypic Characterization

3.5.1 Sequence analysis of the *cyp51* gene

Amplicons of 879, 1,470 and 1,085 bp were obtained as a partial sequence of the *cyp51A* gene using the primers designed by Poloni et al. (2021). After the alignment, a complete sequence of 1,551 bp was obtained for each sequenced isolate. Three haplotypes were identified. All PoT isolates were grouped in the same haplotype (H-1) regardless of their sensitivity to DMI fungicides. Two haplotypes were identified for the non-PoT lineages studied (H-2 and H-3). The isolates grouped in the

H-2 presented 19 point mutation compared to PoT, with 5 non-synonymous substitutions. The H-3 presented one extra non-synonymous substitution compared with H-2. No association between mutations and sensitivity to DMI was found (Table 7).

Table 7. Variations in *cyp51A* gene sequences for four PoT isolates and four non-PoT isolates with different responses to DMI fungicides.

		Phenotype			Nucleotide replacement reference position																			
Isolate code	**Lineage**	**TEBU**	**EPOX**	**Haplotype**	8	231	254	268	270	462	534	546	675	741	773	849	906	927	945	1,002	1,010	1,083	1,155	1,377
UFVPY213	PoT	R	R	H-1	T	T	G	T	A	T	G	C	T	T	G	G	G	C	A	A	G	A	C	A
UFVPY276	PoT	S	S	H-1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
UFVPY311	PoT	R	R	H-1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
UFVPY652	PoT	S	R	H-1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
UFVPY1	non-PoT	S	S	H-2	C	C	A	C	G	C	A	T	C	.	A	C	T	T	G	G	A	G	A	G
UFVPY108	non-PoT	R	R	H-2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
UFVPY112	non-PoT	R	R	H-3	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·
UFVPY200	non-PoT	S	S	H-3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Amino acid replacement					**F 3 S**	-	**R 85 H**	-	-	-	-	-	-	**D 247 E**	**R 258 Q**	-	-	-	-	-	**R 337 Q**	-	**D 385 E**	-

3.5.2 Sequence analysis of the *cytB* gene

A 510 bp fragment where the hot spots 129, 137 and 143 were located were amplified for each isolate. Based on the sequence alignments, all PoT isolates showed the G143A mutation, regardless of their sensitivity to QoI fungicides. On the other hand, none of the non-PoT isolates sequenced showed mutations on the hot spots, regardless of their sensitivity to QoI fungicides. The mutations F129L and G137R were not found in any PoT and non-PoT isolate (Fig 14).

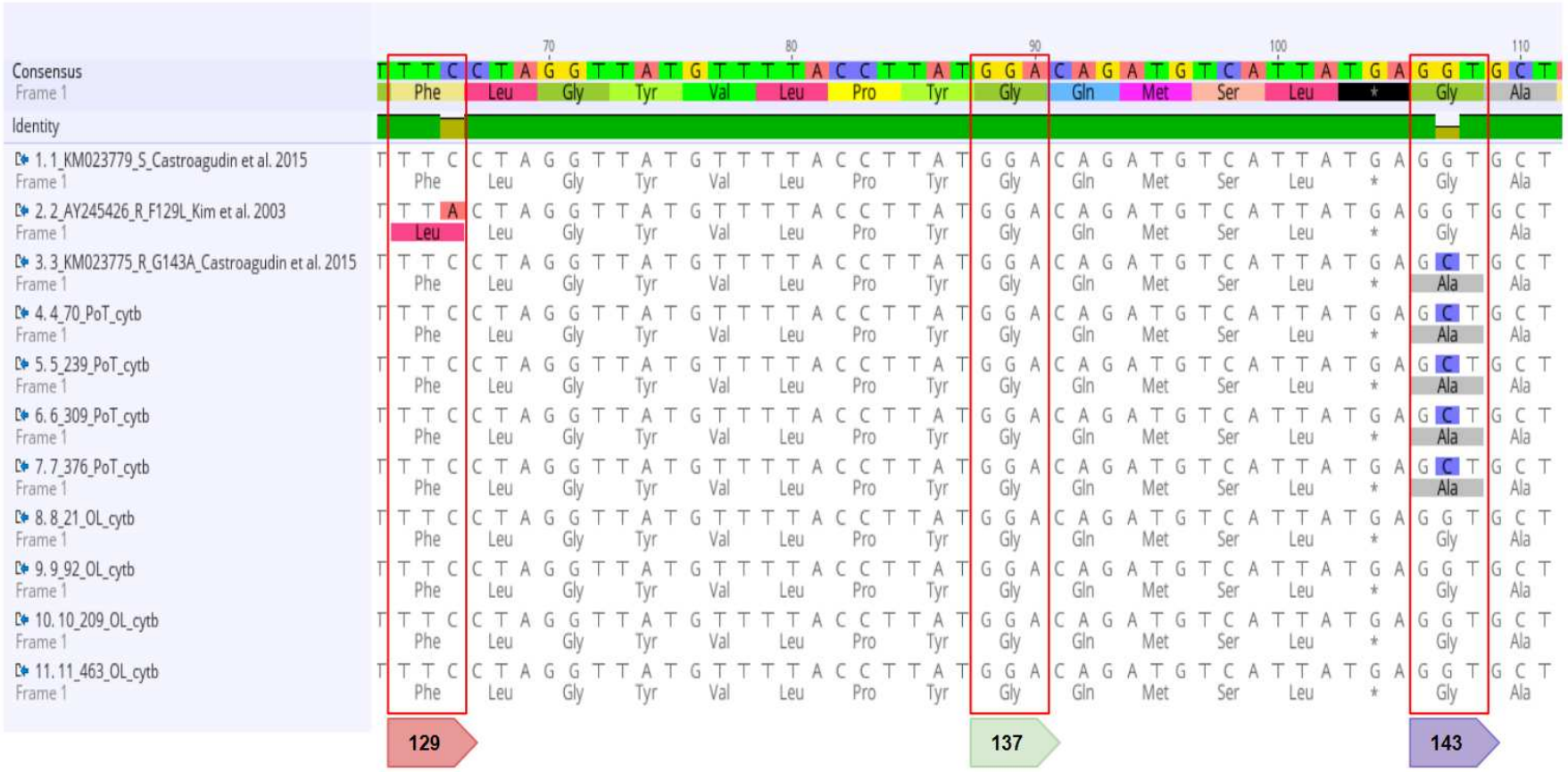

Figure 14. Alignment of amino acid sequences of part of the cytB gene of eleven Pyricularia isolates. The first sequence in the figure (Identity 1.1) corresponds to a sensitive isolate without mutation in the sequence (Castroagudín et al. 2015). The second sequence (Identity 2.2) corresponds to a resistant F129L mutant isolate (Kim et al. 2003). The third sequence (Identity 3.3) corresponds to a resistant G143A mutant isolate (Castroagudín et al. 2015). Identity sequences from 4.4 to 7.7, correspond to PoT isolates studied in this work. Identity sequences from 8.8 to 11.11, correspond to non-PoT isolates studied in this work. The red boxes show the amino acid positions 129, 137 and 143, hot spots for mutations to confer QoI resistance

3.5.3 Sequence analysis of the *sdh* genes

The expected fragment sizes were amplified for each *sdh* subunit. All PoT isolates were grouped in a single haplotype (H-1) when the sequences were analyzed for the different subunits. For the *sdhB* subunit, the non-PoT lineages were grouped into three haplotypes (H-2, H-3 and H-4). The isolates grouped into H-2 presented seven mutations in their sequences compared with PoT isolates. Five of them were involved in amino acid substitutions (non-synonymous mutations). The isolates in H-3 and H-4 presented 6/10 and 7/11 non-synonymous substitutions respectively compared with PoT (Table 8). For *sdhC*, the non-PoT lineages were grouped in three different haplotypes (H-1, H-2 and H-3). The isolate UFVPY92 presented the same sequence of PoT (H-1). The H-2 and H-3 isolates presented just one mutation compared with PoT (synonymous and non-synonymous substitutions respectively) (Table 9). For *sdhD*, non-PoT lineages were grouped in two haplotypes (H-2 and H-3), with 5/6 and 7/7 non-synonymous mutations respectively compared with PoT (Table 9). No association was found between variations in the sequences of the *sdh* genes and sensitivity of SDHI fungicides.

Table 8. Variations in *sdhB* gene sequences for five PoT isolates and four non-PoT isolates with different responses to SDHI fungicides

		Phenotype				Nucleotide replacement reference position											
Isolate code	**Lineage**	**FLUX**	**BENZ**	**BIXA**	**Haplotype**	34	106	183	232	270	287	380	443	500	554	574	672
UFVPY70	PoT	**R**	**R**	**S**	H-1	G	G	G	C	C	G	T	G	C	C	C	A
UFVPY375	PoT	**R**	**R**	**S**	H-1	·	·	·	·	·	·	·	·	·	·	·	·
UFVPY213	PoT	**S**	**S**	**S**	H-1	·	·	·	·	·	·	·	·	·	·	·	·
UFVPY733	PoT	**R**	**R**	**R**	H-1	·	·	·	·	·	·	·	·	·	·	·	·
UFVPY889	PoT	**R**	**S**	**S**	H-1	·	·	·	·	·	·	·	·	·	·	·	·
UFVPY92	non-PoT	**R**	**S**	**S**	H-2	C	A	A	.	T	.	G	A	T	.	.	.
UFVPY120	non-PoT	**S**	**S**	**S**	H-3	G	.	.	T	.	A	.	.	.	G	.	G
UFVPY158	non-PoT	**S**	**R**	**S**	H-4	·	·	·	·	·	·	·	.	·	·	G	·
UFVPY656	non-PoT	**S**	**R**	**S**	H-4	·	·	·	·	·	·	·	·	·	·	·	·
Amino acid replacement						**A 12 P**	**E 36 K**	-	-	-	**R 96 K**	**F 127 C**	**R 148 K**	**T 167 I**	**P 185 R**	**R 192 G**	-

Table 9. Variations in *sdhC* and *sdhD* gene sequences for five PoT isolates and four non-PoT isolates with different responses to SDHI fungicides

					Haplotypes - Nucleotide replacement reference position														
		Phenotype			*sdhC*			*sdhD*											
Isolate code	**Lineage**	**FLUX**	**BENZ**	**BIXA**	**Haplotype**	666	728	**Haplotype**	186	196	205	223	305	404	410	647	680
UFVPY70	PoT	**R**	**R**	**S**	H-1	T	T	H-1	T	T	C	C	A	C	C	T	T
UFVPY375	PoT	**R**	**R**	**S**	H-1	·	·	H-1	·	·	·	·	·	·	·	·	·
UFVPY213	PoT	**S**	**S**	**S**	H-1	·	·	H-1	·	·	·	·	·	·	·	·	·
UFVPY733	PoT	**R**	**R**	**R**	H-1	·	·	H-1	·	·	·	·	·	·	·	·	·
UFVPY889	PoT	**R**	**S**	**S**	H-1	·	·	H-1	·	·	·	·	·	·	·	·	·
UFVPY92	Other	**R**	**S**	**S**	H-1	·	·	H-2	C	G	T	.	.	A	T	C	.
UFVPY120	Other	**S**	**S**	**S**	H-2	C	.	H-2	.	.	.	.	.	.	.	.	.
UFVPY158	Other	**S**	**R**	**S**	H-2	·	·	H-3	T	.	.	T	T	.	C	.	C
UFVPY656	Other	**S**	**R**	**S**	H-3	T	C	H-3	.	.	.	.	.	.	.	.	.
Amino acid replacement							**V 239 A**		**-**	**W 66 G**	**H 69 Y**	**P 75 S**	**K 102 M**	**P 135 Q**	**T 137 M**	**L 216 S**	**M 227 T**

4 DISCUSSION

In this study, 64 isolates of *Pyricularia* obtained from wheat or signal grass plants, collected during 2018 and 2019, were characterized phenotypically and molecularly for their sensitiveness to seven fungicides of three different chemical groups. This is the most comprehensive characterization of fungicide sentivity for the blast pathogen populations obtained in Minas Gerais, the third wheat-producing state in Brazil and where wheat blast epidemics are most frequent. Previous work in the country analyzed *P. oryzae* populations of the *Triticum* lineage mainly obtained from other regions of Brazil (Castroagudín *et al.*, 2015; Oliveira *et al.*, 2015; Casado, 2017; Dorigan *et al.*, 2019; Poloni *et al.*, 2021; Vicentini *et al.*, 2021), including those from the south of Brazil where the control of wheat diseases with fungicides is more intensive compared with Minas Gerais. We found marked differences in the *in vitro* sensitivity among the three chemical groups but also within fungicides of the same group. Overall, our results suggest that multiple resistance to three chemical groups (DMI, QoI and SDHI) is not present in the studied population, contrarily to what has been stated by a series of studies on the topic (Oliveira *et al.*, 2015; Ceresini *et al.*, 2019; Vicentini *et al.*, 2021). Upon inoculation of more or less sensitive representative strains to each fungicide (based on *in vitro* screening), alone or in mixture, we observed that head blast severity was reduced significantly, compared with non-treated plants, regardless of the inoculated strain, when applying a preventative spray of all fungicides but AZOX at the commercial dose.

For DMIs, our results for the *in vitro* assessment of sensitivity are in general agreement with previous works. The EC_{50} values determined for TEBU and EPOX were in a similar range to those reported by Dorigan et al. (2019) and Poloni et al. (2021). The latter authors investigated the resistance to TEBU and EPOX and

concluded, based solely on *in vitro* screening, that the EC_{50} values were indicative of resistance to DMIs in general. However, we showed that EC_{50} values of 1.58 and 1.14 µg/mL for TEBU and EPOX, respectively, in the less sensitive (R) strains inoculated on wheat heads in the greenhouse, were not sufficient to reduce the levels of control by the fungicide (one spray) applied preventatively. In fact, the control efficacy (compared with the check) when applying TEBU was close to 90% regardless of the strain (sensitive or resistant) inoculated. For EPOX, the control efficacy was lower than that for TEBU, ranging from 51.7 (R or R+S) to 83.9 (S) depending on the inoculated strain. Such differences may be explained by the higher dose of TEBU compared with EPOX. The significant association between the EC_{50} values for the two DMIs evaluated suggested a cross resistance mechanism. This is not uncommon for DMIs as shown in previous studies with *Mycosphaerella graminicola, Monilinia fructicola, Colletotrichum spp., Alternaria sp., Cercospora beticola, Fusarium asiaticum and F. graminearum* (Leroux *et al.*, 2007; Yin *et al.*, 2009; Leroux & Walker, 2011; Spolti *et al.*, 2012, 2014; Cools & Fraaije, 2013; Wei *et al.*, 2020; Ishii *et al.*, 2021). The sequence analysis did not show an association between mutations and sensitivity to the different fungicides in the *cyp51A* gene, agreeing with the report by Poloni et al. (2016).

With regards to QoIs, our results for AZOX and PYRA were very contrasting. While AZOX did not inhibit conidia germination even at the maximum dosage (100 µg/ml), an EC_{50} of 1.579 µg/ml on average (0.011 to 3.613 µg/ml) was determined for PYRA. Previously, Castroagudin et al. (2015) reported resistance to AZOX but a few sensitive isolates (5/23 isolates) could be found in their study. Those authors determined 10 µg/ml as discriminatory dose to discriminate the sensitive from the resistant strains. In our study, all PoT isolates were able to germinate more than 50%

at the discriminatory dose of 5 µg/ml, which corroborates previous findings. The mean EC_{50} for PYRA estimated in our work was around 10 times higher than that reported by Oliveira et al. (2015) who studied a collection of 16 PoT isolates from southern Brazil. Direct comparison between our study and those previous works is complicated due to differences in methodology since we used the FRAC-recommended method, the conidial germination assay (FRAC-BR, 2021), and those authors used mycelial growth test. Several authors discuss that the conidial germination method is more appropriate to determine the sensitivity to a fungicide with preventive action, such as QoIs (Olaya *et al.*, 1998; Russell, 2004; Teramoto *et al.*, 2017; Pethybridge *et al.*, 2020; Sautua & Carmona, 2021).

The results of our *in vivo* assays were in partial agreement with those obtained *in vitro*. While the single application of AZOX did not reduce head blast severity (control efficacy of ~20%), application of PYRA resulted in control efficacies that ranged from 73.6 (Resistant strain) to 83.9 (Sensitive strain), depending on the inoculated strain. Based on the *in vitro* screening, cross-resistance and presence of mutations, authors of previous work concluded that QoIs in general should not be used for wheat blast control (Castroagudin et al. 2015; Oliveira *et al.*, 2015; Ceresini *et al.*, 2019; Poloni *et al.*, 2021). Contrarily, we found that one spray of PYRA was sufficient to reduce the disease and spore production at lower levels compared with the non-sprayed plants. The higher toxicity of PYRA compared with AZOX was related previously by other authors for other pathosystems (Mondal *et al.*, 2005; Wise *et al.*, 2008; Hincapie *et al.*, 2014; Sautua & Carmona, 2021)

The analysis of the *cytB* sequences showed the presence of the G143A in the four PoT isolates studied. In *in vitro* experiments, all four showed very low sensitivity to AZOX. Regarding PYRA, two of them were low sensitive and two were high

sensitive. Although the number of isolates sequenced in this work was low, this may be an indication that PYRA resistance may be related with other mechanisms, different to mutations for this particular pathosystem. This can also be inferred based on the different levels of control that AZOX and PYRA showed in the *in vivo* experiments. He *et al.* (2021) obtained similar results in relation to PYRA for *Lasiodiplodia theobromae* in mango. They concluded that the PYRA resistance was not attributable to *cytB* gene alterations for this fungus. They suggested other metabolic resistance mechanisms based on differences in enzyme activity and cell membrane permeability between resistant and sensitive isolates. Despite this, the mechanisms should be studied for this pathosystem. On the other hand, the non-PoT isolates did not present any of the mutations reported as resistance to QoI, regardless of their sensitivity to the fungicides, which may be related to specific characteristics of this different host-specialized population that is less exposed to fungicides in the field compared with PoT.

Marked differences between the three SDHI fungicides were found with regards to the EC_{50} levels; the strains were generally least sensitive to FLUX, followed by BIXA and BENZ. The latter fungicide showed the highest fungitoxicity. Vicentini et al. (2021), using the microtitre plate method to estimate the $EC_{5o,}$ reported low levels of sensitivity to FLUX, in a range similar to our study, for isolates collected from different geographical regions of Brazil in 2018. The most effective fungicide in our study (BENZ) was able to inhibit the *in vitro* conidial germination at very low concentrations (0.001 µg/mL). When comparing the discriminatory doses defined based on the median of the EC_{50} value determined for 13 isolates, the value obtained for BENZ was 800 times lower than that that for BIXA and 2,700 times less than that for FLUX. The high fungitoxicity of BENZ compared with other SDHIs have been

previously reported. For example, in *Colletotrichum* sp. isolates collected from different hosts (Ishii *et al.*, 2016); and also *Sclerotinia sclerotiorum, Venturia inaequalis, Cercospora beticola, Botrytis cinerea and Phakopsora pachyrhizi* (Hu *et al.*, 2016; Villani *et al.*, 2016; Huang *et al.*, 2019; Pethybridge *et al.*, 2020; Müller *et al.*, 2021). Despite the fact that FLUX and BIXA presented low levels of *in vitro* sensitivity (mean EC_{50} values of 36,440 and 9,422 µg/mL respectively), *in vivo* treatments with a single spray of these fungicides effectively reduced disease severity in greenhouse conditions.

For FLUX our results differ from those of Vicentini et al. (2021), since the level of sensitivity of the strain did not affect disease control, where we obtained control efficacies of 30.9 or 46.8% (depending on the experiment), regardless of the sensitivity of the inoculated isolate. In their work, when the plant was inoculated with a resistant isolate, no difference was found between the spray and the non-sprayed plants. A possible explanation for such difference is that we used FLUX at the commercial recommended dose for wheat (50 ml/ha), equivalent to 250 µg/ml in our *in vivo* experiment, while Vicentini et al. (2021) applied a dose of 5 µg/mL, much lower than that recommended for field applications.

The control efficacy in our *in vivo* experiments for BIXA was 48.5% regardless of the sensitivity of the inoculated. The values were slightly higher than obtained for the treatment with FLUX, also the applied dose was too (65 mL/ha).

For BENZ, we found a high concordance between the *in vitro* and *in vivo* experiments. The control efficacy in greenhouse conditions was above 98 and 94% in reduction on severity and spore production respectively using 30 mg/ha, the lowest dose of the SDHI tested in this work. *In vivo* experiments in *B. cinerea* and *C. beticola* demonstrated the high protectant ability of BENZ due to the inhibitory effect

of conidial germination and sporulation (Ishii *et al.*, 2016; He *et al.*, 2018; Pethybridge *et al.*, 2020). Our results contradict the conclusions of Vicentini et al. (2021) who affirmed that PoT populations possess an intrinsic resistance to SDHI fungicides based on studies using FLUX. Because we did not detect correlations between mutations and resistance to fungicides, it is necessary to deepen studies on the difference in the efficacy of fungicides belonging to the same chemical group for this pathosystem. Difference in sensitivity is probably not due to a difference in exposure to the fungitoxic molecule, considering that the three SDHIs tested in this work were introduced in the Brazilian cereal market between the years 2017/18 (AGROFIT, 2021). Although detoxification mechanisms were reported for other pathosystems (Leroux & Walker, 2013; Omrane *et al.*, 2015; Hawkins & Fraaije, 2018), this hypothesis was still not studied for the PoT pathosystem.

An important result of our work is that we did not find a relation between the levels of *in vitro* sensitivity and the levels of disease control. Many of the isolates considered resistant due to their high EC_{50} values, were controlled when inoculated on plants treated with fungicides. This was previously studied for other wheat and soybean pathogens by Reis *et al.* (2015). They concluded that there was no relationship between the EC_{50} and the doses used in commercial applications in the field. They also suggest that *in vitro* experiments should be used just to compare the power among fungicides and to monitor the fungal sensitivity shift towards fungicides, and not to differentiate resistant isolates from sensitive ones. According to our results only AZOX and BENZ that provided extremely high and low EC_{50} values respectively, showed a consistent response in the *in vivo* experiments.

Although control efficiencies under controlled conditions in this work were considerably high, control levels in the field are generally lower even using sequential

sprays. (Ceresini *et al.*, 2019; Singh *et al.*, 2021; Valent *et al.*, 2021). There are many factors that affect the quality of fungicide applications and are directly linked to the disease control efficacy (Reis *et al.*, 2015). The difficulty of reaching the infection site during the application has always been one of the main problems for chemical control in this pathosystem (Maciel, 2011; Ceresini *et al.*, 2019). The sites for penetration of the fungus into the head are mainly the rachis, which are covered by the spikelets. Many authors highlight the need to improve fungicide application technologies such as the type of nozzle, orientation, droplet size and number of impacts in order to fully cover the head (Rocha *et al.*, 2014; Reis *et al.*, 2015; Ceresini *et al.*, 2018; Pizolotto, 2019; Cruz *et al.*, 2019; Valent *et al.*, 2021).

This is the first thorough comparison with regards to fungicide sensitivity with strains obtained from wheat and signal grass, respectively, whose identification was resolved using phylogenetic methods (Ascari et al. unpublished). In general, we observed that the wheat blast pathogen was less sensitive than the signal grass blast pathogen regardless of the relative distance the latter strains were from wheat crops. We studied non-PoT strains (other pathotype or even other species) that were collected hundreds of kms away from wheat in the natural landscape, and which were similarly resistant to AZOX compared with PoT strains, but differently with regards to the other fungicides in *in vitro* experiments. Previous studies have claimed that resistance to DMI and QoIs was pervasive and widespread among pathogen populations obtained from wheat and grasses (Reges *et al.*, 2018, 2019).

Our results demonstrate that blast populations from wheat can be effectively controlled *in vivo* with at least one fungicide of the three chemical groups evaluated, contradicting previous statements. We also conclude that the determination of the EC_{50} *in vitro*, as a sole tool, may not be sufficient to infer loss of efficacy in the field. It

seems necessary to determine control efficacy at commercial doses in *in vivo* experiments. In this regard, we agree with the conclusions by Reis et al. (2015) on the usefulness of *in vitro* experiments to compare fungitoxicity among fungicides but not to infer on control failures in the field, which requires *in vivo* experiments.

Our results highlight good options of fungicides within each chemical groups that can be used for the effectively control the disease in the field, such as PYRA (QoI), TEBU (DMI) and BENZ (SDHI), as long as the application technology ensure the coverage of infection sites at the right time. The efficacy of BENZ was tested for the first time on this pathosystem and we observed a great potential for its inclusion in the management of the disease in the field, due to the high *in vitro* and *in vivo* fungitoxicity. On the other hand, AZOX should not be considered for disease control as previously suggested.

Future works should be focused on: 1) studying the mechanisms involved in fungicide resistance for PYRA and the fungicides of SDHI and DMI groups; 2) studying new fungicide application technologies that guarantee good spray coverage of the rachis tissues; 3) evaluating chemical control strategies in the field that include TEBU, PYRA and BENZ fungicides; 4) determining which EC_{50} values are indicative of resistant or susceptible behavior of the isolates and 5) continuing monitoring of changes in population sensitivity to the main fungicides.

5 REFERENCES

AGROFIT, 2021. Ministério da Agricultura, Pecuária e Abastecimento.

Alberione E, Bainotti C, Cettour I, Salines J, 2008. Evaluación de enfermedades en trigos en siembra de verano en el NEA argentino-Campaña 2007/2008. In: *VII Congreso Nacional de Trigo y V Simposio Nacional de Cereales de Siembra Otoño-Invernal I Encuentro del Mercosur*. 1ª. Santa Rosa, Argentina: UNLPam, 250.

Alves K dos S, 2020. *ec50estimator: An Automated Way to Estimate EC50 for Stratified Datasets*.

Amiri A, Heath SM, Peres NA, 2014. Resistance to Fluopyram, Fluxapyroxad, and Penthiopyrad in *Botrytis cinerea* from Strawberry. *Plant Disease* **98**, 532–539.

Anjos JRN, Silva DB, Charchar MJD, Rodrigues GC, 1996. Occurrence of blast fungus (Pyricularia grisea) on wheat and rye in the savanna region of Central Brazil. *Pesquisa Agropecuária Tropical* **31**, 79–82.

Ascari JP, 2021. Taxonomic and pathogenic diversity of the blast pathogen populations infecting wheat and grasses in Minas Gerais. Viçosa, MG-BRA: Universidad Federal de Viçosa.

Ascari JP, Barro J, Santana FM *et al.*, 2021. Sequential Post-heading Applications for Controlling Wheat Blast: A Nine-year Summary of Fungicide Performance in Brazil. *Plant Disease*.

Aucique-Pérez CE, de Menezes Silva PE, Moreira WR, DaMatta FM, Rodrigues FÁ, 2017. Photosynthesis impairments and excitation energy dissipation on wheat plants supplied with silicon and infected with Pyricularia oryzae. *Plant Physiology and Biochemistry* **121**, 196–205.

Aucique-Pérez CE, Resende RS, Martins AO *et al.*, 2020. How do wheat plants cope

with Pyricularia oryzae infection? A physiological and metabolic approach. *Planta* **252**, 24.

Avenot HF, Michailides TJ, 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Protection* **29**, 643–651.

Barea G, Toledo J, 1996. *Identificación y zonificación de Pyricularia o brusone (Pyricularia oryzae) en el cutivo de trigo en el departamento de Santa Cruz*. Santa Cruz de la Sierra, Bolivia: Centro de Investigación Agrícola Tropical.

Boddy L, 2016. Pathogens of Autotrophs. In: Watkinson SC,, Boddy L,, Money NP, eds. *The Fungi (Third Edition)*. Boston: Academic Press, 245–292.

Cabrera MG, Gutierrez SA, 2007. Primer registro de Pyricularia oryzae en cultivos de trigo del NE de Argentina. In: *Jornada de Actualización en Enfermedades de Trigo*. Lavallol, Buenos Aires: Instituto Fitotécnico de Santa Catalina, 18–180.

Callaway E, 2016. Devastating wheat fungus appears in Asia for first time. *Nature* **532**, 421–422.

Cardoso CA de A, Reis EM, Moreira EN, 2008. Development of a warning system for wheat blast caused by Pyricularia grisea. *Summa Phytopathologica* **34**, 216–221.

Cardozo Téllez L, Chavez A, Bobadilla N, Pérez-Estigarribia P, Kohli M, 2019. Variable resistance of bread wheat (Triticum aestivum) lines carrying 2NS/2AS translocation to wheat blast (H Buerstmayr, Ed,). *Plant Breeding* **138**, 62–68.

Casado P, 2017. Validação do método de microplaca e estudos sobre a resistência ao fungicida fluxapiroxade em populações de *Pyricularia graminis-tritici* no Brasil. Master Thesis Thesis. Ilha Solteira - SP: Universidade Estadual Paulista “Júlio de Mesquita Filho.”

Castroagudín VL, Ceresini PC, de Oliveira SC *et al.*, 2015. Resistance to QoI Fungicides Is Widespread in Brazilian Populations of the Wheat Blast Pathogen *Magnaporthe oryzae*. *Phytopathology* **105**, 284–294.

Castroagudín VL, Moreira SI, Pereira DAS *et al.*, 2016. *Pyricularia graminis-tritici*, a new Pyricularia species causing wheat blast. *Persoonia - Molecular Phylogeny and Evolution of Fungi* **37**, 199–216.

Cecchini G, 2003. Function and Structure of Complex II of the Respiratory Chain. *Annual Review of Biochemistry* **72**, 77–109.

Ceresini PC, Castroagudín VL, Rodrigues FÁ *et al.*, 2018. Wheat blast: Past, present, and future. *Annual Review of Phytopathology* **56**, 427–456.

Ceresini PC, Castroagudín VL, Rodrigues FÁ *et al.*, 2019. Wheat blast: from its origins in South America to its emergence as a global threat: Wheat Blast. *Molecular Plant Pathology* **20**, 155–172.

Coelho MA de O, Torres GAM, Cecon PR, Santana FM, 2016. Sowing date reduces the incidence of wheat blast disease. *Pesquisa Agropecuária Brasileira* **51**, 631–637.

Comissão Brasileira de Pesquisa de Trigo e Triticale, 2020. *Informações Técnicas para Trigo e Triticale*. Passo Fundo, RS. Brasil: Biotrigo Genética.

Cools HJ, Fraaije BA, 2013. Update on mechanisms of azole resistance in *Mycosphaerella graminicola* and implications for future control: Mechanisms of azole resistance in *M. graminicola*. *Pest Management Science* **69**, 150–155.

Couch BC, Fudal I, Lebrun M-H *et al.*, 2005. Origins of host-specific populations of the blast pathogen Magnaporthe oryzae in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. *Genetics* **170**, 613–630.

Cruppe G, Cruz CD, Peterson G *et al.*, 2020. Novel sources of wheat head blast resistance in modern breeding lines and wheat wild relatives. *Plant Disease* **104**, 35–43.

Cruz CD, Bockus WW, Stack JP, Valent B, Maciel JN, Peterson GL, 2016a. A standardized inoculation protocol to test wheat cultivars for reaction to head blast caused by Magnaporthe oryzae (Triticum pathotype). *Plant Health Progress* **17**, 186–187.

Cruz MFA da, Diniz APC, Rodrigues FA, Barros EG de, 2011a. Aplicação foliar de produtos na redução da severidade da brusone do trigo. *Tropical Plant Pathology* **36**, 424–428.

Cruz MFA da, Diniz APC, Rodrigues FA, Barros EG de, 2011b. Aplicação foliar de produtos na redução da severidade da brusone do trigo. *Tropical Plant Pathology* **36**, 424–428.

Cruz CD, Kiyuna J, Bockus WW, Todd TC, Stack JP, Valent B, 2015a. Magnaporthe oryzae conidia on basal wheat leaves as a potential source of wheat blast inoculum. *Plant Pathology* **64**, 1491–1498.

Cruz CD, Magarey RD, Christie DN *et al.*, 2016b. Climate Suitability for *Magnaporthe oryzae Triticum* Pathotype in the United States. *Plant Disease* **100**, 1979–1987.

Cruz CD, Peterson GL, Bockus WW *et al.*, 2016c. The 2NS translocation from Aegilops ventricosa confers resistance to the Triticum pathotype of Magnaporthe oryzae. *Crop Science* **56**, 990–1000.

Cruz MFA, Prestes AM, Maciel JLN, Scheeren PL, 2010. Partial resistance to blast on common and synthetic wheat genotypes in seedling and in adult plant growth stages [Resistência parcial à brusone de genótipos de trigo comum e

sintético nos estádios de planta jovem e de planta adulta]. *Tropical Plant Pathology* **35**, 024–031.

Cruz MFA, Rios JA, Araujo L, Ávila Rodrigues F, 2016d. Infection process of Pyricularia oryzae on the leaves of wheat seedlings. *Tropical Plant Pathology* **41**, 123–127.

Cruz CD, Santana FM, Todd TC *et al.*, 2019. Multi-environment assessment of fungicide performance for managing wheat head blast (WHB) in Brazil and Bolivia. *Tropical Plant Pathology* **44**, 183–191.

Cruz MFA da, Silva LAF, Rios JA, Debona D, Rodrigues FÁ, 2015b. Microscopic aspects of the colonization of Pyricularia oryzae on the rachis of wheat plants supplied with silicon. *Bragantia* **74**, 207–214.

Cruz CD, Valent B, 2017. Wheat blast disease: danger on the move. *Tropical Plant Pathology* **42**, 210–222.

Danelli ALD, Fernandes JMC, Maciel JLN, Boaretto C, Forcelini CA, 2019. Monitoring Pyricularia sp. airborne inoculum in Passo Fundo, Rio Grande do Sul, Brazil. *Summa Phytopathologica* **45**, 361–367.

D'Ávila LS, Filippi MCCD, Café-Filho AC, 2021. Sensitivity of Pyricularia oryzae populations to fungicides over a 26-year time frame in Brazil. *Plant Disease*.

Debona D, Cruz MFA, Rodrigues FA, 2017. Calcium-triggered accumulation of defense-related transcripts enhances wheat resistance to leaf blast. *Tropical Plant Pathology* **42**, 309–314.

Debona D, Rios JA, Nascimento KJT, Silva LC, Rodrigues FA, 2016. Influence of magnesium on physiological responses of wheat infected by Pyricularia oryzae. *Plant Pathology* **65**, 114–123.

Debona D, Rodrigues FA, Rios JA, Nascimento KJT, Silva LC, 2014. The effect of

silicon on antioxidant metabolism of wheat leaves infected by Pyricularia oryzae. *Plant Pathology* **63**, 581–589.

Dhingra OD, Sinclair JB, 1995. *Basic Plant Pathology Methods*. Boca Raton: CRC Press.

Dorigan AF, Carvalho GD, Poloni NM, Negrisoli MM, Maciel JLN, Ceresini PC, 2019. Resistance to triazole fungicides in *Pyricularia* species associated with invasive plants from wheat fields in Brazil. *Acta Scientiarum. Agronomy* **41**, 1–10.

Ebbole DJ, 2007. *Magnaporthe* as a model for understanding host-pathogen interactions. *Annual Review of Phytopathology* **45**, 437–456.

Farman M, Peterson G, Chen L *et al.*, 2017. The *Lolium* Pathotype of *Magnaporthe oryzae* Recovered from a Single Blasted Wheat Plant in the United States. *Plant Disease* **101**, 684–692.

Fernandez J, Orth K, 2018. Rise of a cereal killer: The biology of *Magnaporthe oryzae* biotrophic growth. *Trends in microbiology* **26**, 582–597.

FRAC-BR, 2021. Comitê de Ação a Resistência a Fungicidas. *frac-br*.

Gomes DP, Rocha VS, Rocha JR do AS de C, Pereira OL, Souza MA de, 2018. Potential of transmission of Pyricularia graminis-tritici from plant to seed and from seed to seedling in wheat genotypes with different degrees of blast resistance. *Journal of Seed Science* **40**, 16–24.

Gomes DP, Rocha VS, Rocha J, de Souza MA, Pereira OL, 2019. Temporal progression of wheat blast as a function of primary inoculum, fungicide application and genotype resistance [Progresso temporal da brusone do trigo em função do inóculo primário, da aplicação de fungicida e da resistência dos genótipos]. *Summa Phytopathologica* **45**, 50–58.

Gongora-Canul C, Salgado JD, Singh D *et al.*, 2020. Temporal Dynamics of Wheat Blast Epidemics and Disease Measurements Using Multispectral Imagery. *Phytopathology*^{TM}**110**, 393–405.

Goulart ACP, Paiva FA, 1990. Transmission of Pyricularia oryzae by wheat (Triticum aestivum) seeds. **15**, 359–362.

Goulart ACP, Paiva F de A, 2000. *Avaliação de Perdas no Rendimento de Grãos de Trigo Causadas por Pyricularia grisea, no Período de 1988 a 1992, em Mato Grosso do Sul*. Dourados/MS: Embrapa.

Goulart ACP, Paiva F, Mesquita AN, 1990. Occurrence and losses caused by wheat (Triticum aestivum L.) blast (Pyricularia oryzae Cav.) in the state of Mato Grosso do Sul, in 1988. In: *Annual Wheat Newsletter*. Fort Collins: Department of Agronomy, Colorado State University,.

Goulart ACP, Sousa PG, Urashima AS, 2007. Danos em trigo causados pela infecção de Pyricularia grisea. *Summa Phytopathologica* **33**, 358–363.

Hall TA, 1999. *BioEdit: a user-friendly biological sequence alignment editor and analysis program*.

Hartig F, 2021. *Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*.

Hawkins NJ, Fraaije BA, 2018. Fitness Penalties in the Evolution of Fungicide Resistance. *Annual Review of Phytopathology* **56**, 339–360.

He L, Cui K, Song Y, Mu W, Liu F, 2018. High-Efficiency Control of Gray Mold by the Novel SDHI Fungicide Benzovindiflupyr Combined with a Reasonable Application Approach of Dipping Flower. *Journal of Agricultural and Food Chemistry* **66**, 6692–6698.

He R, Yang Y, Hu Z, Xue R, Hu Y, 2021. Resistance mechanisms and fitness of

pyraclostrobin-resistant isolates of Lasiodiplodia theobromae from mango orchards (AK Pandey, Ed,). *PLOS ONE* **16**, e0253659.

Hincapie M, Wang N-Y, Peres NA, Dewdney MM, 2014. Baseline Sensitivity of *Guignardia citricarpa* Isolates from Florida to Azoxystrobin and Pyraclostrobin. *Plant Disease* **98**, 780–789.

Hirata K, Kusaba M, Chuma I *et al.*, 2007. Speciation in Pyricularia inferred from multilocus phylogenetic analysis. *Mycological Research* **111**, 799–808.

Hollomon DW, 2015. Fungicide Resistance: Facing the Challenge. **51**, 7.

Hothorn T, Bretz F, Westfall P, 2008. Simultaneous inference in general parametric models. *Biometrical Journal. Biometrische Zeitschrift* **50**, 346–363.

Hu M-J, Fernández-Ortuño D, Schnabel G, 2016. Monitoring Resistance to SDHI Fungicides in *Botrytis cinerea* From Strawberry Fields. *Plant Disease* **100**, 959–965.

Huang X, luo jian, Song Y, Li B, Mu W, Liu F, 2019. Favorable bioactivity of the SDHI fungicide benzovindiflupyr against Sclerotinia sclerotiorum mycelial growth, sclerotial production, and myceliogenic and carpogenic germination of sclerotia. *Plant Disease*.

Huber DM, Thompson IA, 2007. Nitrogen and plant disease. In: Datnoff LE,, Datnoff WH,, Huber DM, eds. *Mineral Nutrition and Plant Disease*. APS Press: APS Press, 31–44.

Igarashi S, Utiamada CM, Igarashi LC, Kazuma AH, Lopes RS, 1986. Occurrence of *Pyricularia* sp. in wheat (*Triticum aestivum* L.) in the State of Parana, Brazil. *Fitopatologia Brasileira* **11**, 351–352.

Integrated DNA Technologies OligoAnalyzer - RRID:SCR_001363 *OligoAnalyzer™ Tool*. © 2021 Integrated DNA Technologies, Inc.

Ishii H, Bryson PK, Kayamori M, Miyamoto T, Yamaoka Y, Schnabel G, 2021. Cross-resistance to the new fungicide mefentrifluconazole in DMI-resistant fungal pathogens. *Pesticide Biochemistry and Physiology* **171**, 104737.

Ishii H, Fountaine J, Chung W-H *et al.*, 2009. Characterisation of QoI-resistant field isolates of *Botrytis cinerea* from citrus and strawberry: QoI-resistant isolates of *B. cinerea*. *Pest Management Science* **65**, 916–922.

Ishii H, Hollomon DW (Eds.), 2015. *Fungicide Resistance in Plant Pathogens*. Tokyo: Springer Japan.

Ishii H, Zhen F, Hu M, Li X, Schnabel G, 2016. Efficacy of SDHI fungicides, including benzovindiflupyr, against *Colletotrichum* species: Efficacy of benzovindiflupyr against *Colletotrichum* species. *Pest Management Science* **72**, 1844–1853.

Islam MT, Croll D, Gladieux P *et al.*, 2016. Emergence of wheat blast in Bangladesh was caused by a South American lineage of Magnaporthe oryzae. *BMC Biology* **14**, 84.

Islam MT, Kim K-H, Choi J, 2019. Wheat Blast in Bangladesh: The Current Situation and Future Impacts. *The Plant Pathology Journal* **35**, 1–10.

Jank L, Barrios SC, Valle CB do, Simeão RM, Alves GF, 2014. The value of improved pastures to Brazilian beef production. *Crop and Pasture Science* **65**, 1132–1137.

Jéssica Rosset Ferreira, Vancini C, Deuner CC *et al.*, 2018. Absence of 2NS/2AS in Wheat Resistance Sources to Magnaporthe oryzae in Brazil.

Kearse M, Moir R, Wilson A *et al.*, 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649.

Kikuhara K, Iiyama K, Matsumoto M, Furuya N, 2019. First report on DMI fungicide

resistance in Gymnosporangium asiaticum, the causal agent of Japanese pear rust, in Japan. *Journal of General Plant Pathology* **85**, 49–56.

Kim Y-S, Dixon EW, Vincelli P, Farman ML, 2003. Field Resistance to Strobilurin (Q o I) Fungicides in *Pyricularia grisea* Caused by Mutations in the Mitochondrial Cytochrome b Gene. *Phytopathology* **93**, 891–900.

Kohli MM, Mehta YR, Guzman E, De Viedma L, Cubilla LE, 2011. *Pyricularia* blast – a threat to wheat cultivation. *Czech Journal of Genetics and Plant Breeding* **47**, S130–S134.

Kõressaar T, Lepamets M, Kaplinski L, Raime K, Andreson R, Remm M, 2018. Primer3_masker: integrating masking of template sequence with primer design software (J Hancock, Ed,). *Bioinformatics* **34**, 1937–1938.

Kovaleski M, Maciel JLN, Santos GB dos, Silva AN da, Deuner CC, 2020. Conidia sporulation of Pyricularia oryzae in segments of wheat plants under six different temperatures. *Ciência Rural* **50**, e20190573.

Leach CM, 1980. Influence of Humidity, Red-Infrared Radiation, and Vibration on Spore Discharge by *Pyricularia oryzae*. *Phytopathology* **70**, 201–205.

Lenth R, Buerkner P, Herve M, Love J, Riebl H, Singmann H, 2020. *emmeans: Estimated Marginal Means, aka Least-Squares Means*.

Leroux P, Albertini C, Gautier A, Gredt M, Walker A-S, 2007. Mutations in theCYP51 gene correlated with changes in sensitivity to sterol 14α-demethylation inhibitors in field isolates ofMycosphaerella graminicola. *Pest Management Science* **63**, 688–698.

Leroux P, Gredt M, Leroch M, Walker A-S, 2010. Exploring Mechanisms of Resistance to Respiratory Inhibitors in Field Strains of *Botrytis cinerea* , the Causal Agent of Gray Mold. *Applied and Environmental Microbiology* **76**,

6615–6630.

Leroux P, Walker A-S, 2011. Multiple mechanisms account for resistance to sterol 14α-demethylation inhibitors in field isolates of Mycosphaerella graminicola. *Pest Management Science* **67**, 44–59.

Leroux P, Walker A-S, 2013. Activity of fungicides and modulators of membrane drug transporters in field strains of Botrytis cinerea displaying multidrug resistance. *European Journal of Plant Pathology* **135**, 683–693.

Maciel J, 2011. Magnaporthe oryzae, the blast pathogen: current status and options for its control. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **6**.

Maciel JLN, Ceresini PC, Castroagudin VL, Zala M, Kema GHJ, McDonald BA, 2014. Population Structure and Pathotype Diversity of the Wheat Blast Pathogen *Magnaporthe oryzae* 25 Years After Its Emergence in Brazil. *Phytopathology* **104**, 95–107.

Maciel JLN, Danelli ALD, Boaretto C, Forcelini CA, 2013. Diagrammatic scale for the assessment of blast on wheat spikes. *Summa Phytopathologica* **39**, 162–166.

Mair WJ, Thomas GJ, Dodhia K *et al.*, 2019. *Parallel evolution of multiple mechanisms for demethylase inhibitor fungicide resistance in the barley pathogen* Pyrenophora teres *f. sp.* maculata. Plant Biology.

Malaker PK, Barma NCD, Tiwari TP *et al.*, 2016. First Report of wheat blast caused by Magnaporthe oryzae pathotype Triticum in Bangladesh. *Plant Disease* **100**, 2330.

Martinez SI, Wegner A, Bohnert S, Schaffrath U, Perelló A, 2021. Tracing seed to seedling transmission of the wheat blast pathogen Magnaporthe oryzae pathotype Triticum. *Plant Pathology*.

Mills KB, Salgado JD, Cruz CD, Valent B, Madden LV, Paul PA, 2020. Comparing the Temporal Development of Wheat Spike Blast Epidemics in a Region of Bolivia Where the Disease Is Endemic. *Plant Disease*, PDIS-04-20-0876.

Mondal SN, Bhatia A, Shilts T, Timmer LW, 2005. Baseline Sensitivities of Fungal Pathogens of Fruit and Foliage of Citrus to Azoxystrobin, Pyraclostrobin, and Fenbuconazole. *Plant Disease* **89**, 1186–1194.

Moreira C, Camacho MA, Graichen FAS, 2020. Redução da severidade da brusone do trigo com aplicação foliar de sulfato de zinco. *Summa Phytopathologica* **46**, 255–259.

Müller MA, Stammler G, May De Mio LL, 2021. Multiple resistance to DMI, QoI and SDHI fungicides in field isolates of Phakopsora pachyrhizi. *Crop Protection* **145**, 105618.

Oh HS, Tosa Y, Takabayashi N *et al.*, 2002. Characterization of an *Avena* isolate of *Magnaporthe grisea* and identification of a locus conditioning its specificity on oat. *Canadian Journal of Botany* **80**, 1088–1095.

Olaya G, Zheng D, Köller W, 1998. Differential Responses of GerminatingVenturiainaequalis Conidia to Kresoxim-methyl. *Pestic. Sci.* **54**, 7.

Oliveira SC de, Castroagudín VL, Maciel JLN, Pereira DA dos S, Ceresini PC, 2015. Resistência cruzada aos fungicidas QoI azoxistrobina e piraclostrobina no patógeno da brusone do trigo Pyricularia oryzae no Brasil. *Summa Phytopathologica* **41**, 298–304.

Omrane S, Sghyer H, Audéon C *et al.*, 2015. Fungicide efflux and the MgMFS1 transporter contribute to the multidrug resistance phenotype in *Z ymoseptoria tritici* field isolates: Fungicide efflux & *MgMFS 1* contribute to MDR in *Z. tritici*. *Environmental Microbiology* **17**, 2805–2823.

Pagani APS, Dianese AC, Café-Filho AC, 2014. Management of wheat blast with synthetic fungicides, partial resistance and silicate and phosphate minerals. *Phytoparasitica* **42**, 609–617.

Pak D, You MP, Lanoiselet V, Barbetti MJ, 2021. Management of rice blast (Pyricularia oryzae): implications of alternative hosts. *European Journal of Plant Pathology*.

Peng R, 2019. *simpleboot: Simple bootstrap routines*.

Perelló A, Martínez I, Molina M, 2015. First Report of Virulence and Effects of Magnaporthe oryzae Isolates Causing Wheat Blast in Argentina. *Plant Disease* **99**, 1177.

Pethybridge SJ, Sharma S, Hansen ZR, Vaghefi N, Hanson LE, Kikkert JR, 2020. Improving fungicide-based management of Cercospora leaf spot in table beet in New York, USA. *Canadian Journal of Plant Pathology* **42**, 353–366.

Pizolotto CA, 2019. Aspectos epidemiológicos e manejo integrado da brusone do trigo. Doctoral Thesis Thesis. Brazil: Uneversidade de Passo Fundo.

Pizolotto CA, Maciel JLN, Fernandes JMC, Boller W, 2019. Saprotrophic survival of Magnaporthe oryzae in infested wheat residues. *European Journal of Plant Pathology* **153**, 327–339.

Poloni NM, Carvalho G, Vicentini S *et al.*, 2021. Widespread distribution of resistance to triazole fungicides in Brazilian populations of the wheat blast pathogen. *Plant Pathology* **70**, 436–448.

Prabhu AS, Filippi MC, Castro N, 1992. Pathogenic variation among isolates of Pyricularia oryzae affecting rice, wheat, and grasses in Brazil. *Tropical Pest Management* **38**, 367–371.

R Core Team, 2021. *R: A Language and Environment for Statistical Computing*.

Vienna, Austria: R Foundation for Statistical Computing.

de Ramón-Carbonell M, Sánchez-Torres P, 2020. Significance of 195 bp-enhancer of PdCYP51B in the acquisition of Penicillium digitatum DMI resistance and increase of fungal virulence. *Pesticide Biochemistry and Physiology* **165**, 104522.

Reges JT de A, Jesus MN de, Silva SDR da, Souza MH de, Rodrigues JW, 2019. Teste de patogenicidade dos isolados de Pyricularia oryzae nos hospedeiros de trigo, cevada, arroz e braquiária. *Revista Cultura Agronômica* **28**, 19–28.

Reges JT de A, Santos I de J, Rodrigues JW, Souza MH de, Silva SDR da, Jesus MN de, 2018. Caracterização fenotípica de isolados de Pyricularia oryzae de trigo e plantas invasoras. *Revista de Ciências Agrárias* **61**.

Reis EM, Zanatta M, Carmona M, Menten JOM, 2015. Relationship between IC50determined in vitro/in vivoand the fungicide rate used in the Field. *Summa Phytopathologica* **41**, 49–53.

Rios JA, Rios VS, Paul PA, Souza MA, Araujo L, Rodrigues FA, 2016. Fungicide and cultivar effects on the development and temporal progress of wheat blast under field conditions. *Crop Protection* **89**, 152–160.

Rios JA, Rios VS, Paul PA, Souza MA, Neto LBMC, Rodrigues FA, 2017. Effects of blast on components of wheat physiology and grain yield as influenced by fungicide treatment and host resistance. *Plant Pathology* **66**, 877–889.

Ritz C, Baty F, Streibig JC, Gerhard D, 2015. Dose-Response Analysis Using R (Y Xia, Ed,). *PLOS ONE* **10**, e0146021.

Rocha JR do AS de C, de Paula IG, Gloria HB, Pimentel AJB, Ribeiro G, de Souza MA, 2019. Screening wheat genotypes for resistance to wheat blast disease in the vegetative and reproductive stages. *Euphytica* **215**.

Rocha JR do AS de C, Pimentel AJB, Ribeiro G, Souza MA de, 2014. Eficiência de fungicidas no controle da brusone em trigo. *Summa Phytopathologica* **40**, 347–352.

Rodrigues FÁ, Rios JA, Debona D, Aucique-Pérez CE, 2017. Pyricularia oryzae-wheat interaction: physiological changes and disease management using mineral nutrition and fungicides. *Tropical Plant Pathology* **42**, 223–229.

Roy KK, Reza MMA, Muzahid-E-Rahman M *et al.*, 2021. Evaluation of elite bread wheat lines for resistance to blast disease in Bangladesh. *Euphytica* **217**, 151.

Russell PE, 2004. SENSITIVITY BASELINES IN FUNGICIDE RESISTANCE RESEARCH AND MANAGEMENT. *FRAC Monograph, 3. Brussels: CropLife International* **3**, 60.

Sadat MdA, Choi J, 2017. Wheat Blast: A New Fungal Inhabitant to Bangladesh Threatening World Wheat Production. *The Plant Pathology Journal* **33**, 103–108.

Santana FM, Lau D, Sbalcheiro CC *et al.*, 2016. *Eficiência de fungicidas para o controle da brusone do trigo: resultados dos ensaios cooperativos – safra 2014*. Passo Fundo, RS. Brasil: Embrapa Trigo.

Sautua FJ, Carmona MA, 2021. Detection and characterization of QoI resistance in Pyrenophora tritici-repentis populations causing tan spot of wheat in Argentina. *Plant Pathology Journal* **70**, 12.

Silva GB da, Prabhu AS, 2005. Quantificação de conídios de Pyricularia grisea no plantio direto e convencional de arroz de terras altas. *Fitopatologia Brasileira* **30**, 569–573.

Silva ET, Rios JA, Araujo MUP, Silveira PR, Rodrigues FA, 2019. Defence responses in flag leaves and spikes of common wheat *Triticum aestivum* cultivars with

contrasting levels of basal resistance to blast caused by *Pyricularia oryzae*. *Plant Pathology* **68**, 645–658.

Singh PK, Gahtyari NC, Roy C *et al.*, 2021. Wheat blast: A disease spreading by intercontinental jumps and its management strategies. *Frontiers in Plant Science* **12**, 1–21.

Spolti P, Del Ponte EM, Dong Y, Cummings JA, Bergstrom GC, 2014. Triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York wheat and competitiveness of a tebuconazole-resistant isolate. *Plant Disease* **98**, 607–613.

Spolti P, Jorge BC de, Del Ponte EM, 2012. Sensitivity of *Fusarium graminearum* causing head blight of wheat in Brazil to tebuconazole and metconazole fungicides. *Tropical Plant Pathology* **37**, 419–423.

Surovy MZ, Mahmud NU, Bhattacharjee P *et al.*, 2020. Modulation of Nutritional and Biochemical Properties of Wheat Grains Infected by Blast Fungus Magnaporthe oryzae Triticum Pathotype. *Frontiers in Microbiology* **11**.

Tanaka M, Nakayashiki H, Tosa Y, 2009. Population structure of Eleusine isolates of Pyricularia oryzae and its evolutionary implications. *Journal of General Plant Pathology* **75**, 173–180.

Tembo B, 2019. A Review of Rain-Fed Wheat Production Constraints in Zambia. *Journal of Agriculture and Crops*, 158–161.

Tembo B, Mulenga RM, Sichilima S *et al.*, 2020. Detection and characterization of fungus (Magnaporthe oryzae pathotype Triticum) causing wheat blast disease on rain-fed grown wheat (Triticum aestivum L.) in Zambia. *PLOS ONE* **15**, 1–10.

Teramoto A, Meyer MC, Suassuna ND, Cunha MG da, 2017. In vitro sensitivity of

Corynespora cassiicola isolated from soybean to fungicides and field chemical control of target spot. *Summa Phytopathologica* **43**, 281–289.

Thierry M, Chatet A, Fournier E, Tharreau D, Ioos R, 2020. A PCR, qPCR, and LAMP Toolkit for the Detection of the Wheat Blast Pathogen in Seeds. *Plants* **9**, 277.

Tormen NR, Lenz G, Minuzzi SG, Uebel JD, Cezar HS, Balardin RS, 2013. Reação de cultivares de trigo à ferrugem da folha e mancha amarela e responsividade a fungicidas. *Ciência Rural* **43**, 239–246.

Tosa Y, Chuma I, 2014. Classification and parasitic specialization of blast fungi. *Journal of General Plant Pathology* **80**, 202–209.

Tosa Y, Hirata K, Tamba H *et al.*, 2004. Genetic Constitution and Pathogenicity of *Lolium* Isolates of *Magnaporthe oryzae* in Comparison with Host Species-Specific Pathotypes of the Blast Fungus. *Phytopathology* **94**, 454–462.

Tosa Y, Tamba H, Tanaka K, Mayama S, 2006. Genetic Analysis of Host Species Specificity of *Magnaporthe oryzae* Isolates from Rice and Wheat. *Phytopathology* **96**, 480–484.

Tucker MA, Lopez‐Ruiz F, Cools HJ, Mullins JG, Jayasena K, Oliver RP, 2020. Analysis of mutations in West Australian populations of *Blumeria graminis* f. sp. *hordei CYP51* conferring resistance to DMI fungicides. *Pest Management Science* **76**, 1265–1272.

Urashima AS, Alves AF, Silva FN, Oliveira D, Gazaffi R, 2017. Host range, mating type and population structure of *Magnaporthe* sp. of a single barley field in São Paulo state, Brazil. *Journal of Phytopathology* **165**, 414–424.

Urashima AS, Grosso CRF, Stabili A *et al.*, 2009. Effect of Magnaporthe grisea on

Seed Germination, Yield and Quality of Wheat. In: Wang G-L,, Valent B, eds. *Advances in Genetics, Genomics and Control of Rice Blast Disease*. Dordrecht: Springer Netherlands, 267–277.

Urashima AS, Igarashi S, Kato H, 1993. Host range, mating type, and fertility of Pyricularia grisea from wheat in Brazil. *Plant Disease* **77**, 1211–1216.

Urashima AS, Leite SF, Galbieri R, 2007. Eficiência da disseminação aérea em Pyricularia grisea. *Summa Phytopathologica* **33**, 275–279.

Valent B, Cruppe G, Stack JP *et al.*, 2021. Recovery Plan for Wheat Blast Caused by Magnaporthe oryzae Pathotype Triticum. *Plant Health Progress*, PHP-11-20-0101-RP.

Vicentini SNC, Casado PS, Carvalho G *et al.*, 2021. Monitoring of Brazilian wheat blast field populations reveals resistance to QoI, DMI, and SDHI fungicides. *Plant Pathology*, ppa.13470.

Viedma LQ, 2005. Wheat blast occurrence in Paraguay (abstract). *Phytopathology*, S152.

Villani SM, Ayer K, Cox KD, 2016. Molecular Characterization of the *sdhB* Gene and Baseline Sensitivity to Penthiopyrad, Fluopyram, and Benzovindiflupyr in *Venturia inaequalis*. *Plant Disease* **100**, 1709–1716.

Wei L, Chen W, Zhao W *et al.*, 2020. Mutations and Overexpression of *CYP51* Associated with DMI-Resistance in *Colletotrichum gloeosporioides* from Chili. *Plant Disease* **104**, 668–676.

Wilson RA, Talbot NJ, 2009. Under pressure: investigating the biology of plant infection by Magnaporthe oryzae. *Nature Reviews Microbiology* **7**, 185–195.

Wise KA, Bradley CA, Pasche JS, Gudmestad NC, Dugan FM, Chen W, 2008. Baseline Sensitivity of *Ascochyta rabiei* to Azoxystrobin, Pyraclostrobin, and

Boscalid. *Plant Disease* **92**, 295–300.

Wyand RA, Brown JKM, 2005. Sequence variation in the CYP51 gene of Blumeria graminis associated with resistance to sterol demethylase inhibiting fungicides. *Fungal Genetics and Biology* **42**, 726–735.

Xavier Filha MS, Rodrigues FA, Domiciano GP, Oliveira HV, Silveira PR, Moreira WR, 2011. Wheat resistance to leaf blast mediated by silicon. *Australasian Plant Pathology* **40**, 28–38.

Yamashita M, Fraaije B, 2018. Non‐target site SDHI resistance is present as standing genetic variation in field populations of *Zymoseptoria tritici*. *Pest Management Science* **74**, 672–681.

Yesmin N, Jenny F, Abdullah HM *et al.*, 2020. A review on South Asian wheat blast: The present status and future perspective. *Plant Pathology* **69**, 1618–1629.

Yin Y, Liu X, Li B, Ma Z, 2009. Characterization of Sterol Demethylation Inhibitor-Resistant Isolates of *Fusarium asiaticum* and *F. graminearum* Collected from Wheat in China. *Phytopathology*® **99**, 487–497.

Zadoks JC, Chang TT, Konzak CF, 1974. A decimal code for the growth stages of cereals. *Weed Research* **14**, 415–421.

6 SUPPLEMENTARY MATERIAL

S.1. EC_{50} values of PoT (C17 +) and non-PoT (C17 -) isolates for DMI fungicides obtained from *in vitro* characterization tests.

Isolate Code	City	Region	Year	Host	C17	EC50 (µg/mL)	
						TEBU	EPOX
UFVPY1	Uberaba	TM	2018	*Panicum maximum*	-	0.152	0.045
UFVPY21	Madre de Deus	Centro Sul	2018	*Urochloa* sp.	-	0.331	0.133
UFVPY70	Viçosa	Centro Sul	2018	*Triticum aestivum*	+	1.033	0.683
UFVPY92	Caxambú	Centro Sul	2018	*Urochloa* sp.	-	0.872	0.331
UFVPY108	Patos de Minas	TM	2018	*Urochloa* sp.	-	1.285	0.850
UFVPY109	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.784	0.503
UFVPY110	Patos de Minas	TM	2018	*Urochloa* sp.	-	1.049	0.946
UFVPY112	Patos de Minas	TM	2018	*Urochloa* sp.	-	1.142	0.855
UFVPY118	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.774	0.578
UFVPY119	Patos de Minas	TM	2018	*Urochloa* sp.	-	1.307	0.819
UFVPY120	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.587	0.379
UFVPY121	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.503	0.296
UFVPY135	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.554	0.339
UFVPY158	Uberaba	TM	2018	*Urochloa* sp.	-	1.071	0.559
UFVPY165	Uberaba	TM	2018	*Triticum aestivum*	+	1.038	0.641
UFVPY166	Uberaba	TM	2018	*Urochloa* sp.	-	1.066	0.478
UFVPY167	Patos de Minas	TM	2018	*Triticum aestivum*	+	1.199	0.628
UFVPY168	Patos de Minas	TM	2018	*Triticum aestivum*	+	1.438	0.721
UFVPY171	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.441	0.284
UFVPY196	Campos Altos	TM	2019	*Urochloa* sp.	-	0.619	0.235
UFVPY197	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.317	0.109
UFVPY200	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.353	0.003
UFVPY203	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.354	0.078
UFVPY209	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.400	0.153
UFVPY210	Santana da	Centro Sul	2019	*Panicum*	-	0.319	0.259

	Vargem			*maximum*			
UFVPY213	S. Gonçalo do Pará	Centro Sul	2019	*Urochloa* sp.	+	1.432	1.144
UFVPY221	Ibiá	TM	2019	*Triticum aestivum*	+	0.963	0.517
UFVPY224	Ibiá	TM	2019	*Triticum aestivum*	+	0.921	0.714
UFVPY238	Ibiá	TM	2019	*Triticum aestivum*	+	0.835	0.540
UFVPY239	Ibiá	TM	2019	*Triticum aestivum*	+	1.016	0.530
UFVPY255	Santa Juliana	TM	2019	*Triticum aestivum*	+	1.253	0.676
UFVPY270	Campos Altos	TM	2019	*Triticum aestivum*	+	1.546	1.093
UFVPY271	Campos Altos	TM	2019	*Triticum aestivum*	+	1.213	0.848
UFVPY276	Campos Altos	TM	2019	*Triticum aestivum*	+	0.647	0.200
UFVPY309	Boa Esperança	Centro Sul	2019	*Triticum aestivum*	+	1.512	0.862
UFVPY311	Boa Esperança	Centro Sul	2018	*Triticum aestivum*	+	1.577	1.101
UFVPY312	Boa Esperança	Centro Sul	2018	*Triticum aestivum*	+	1.260	0.699
UFVPY360	Patos de Minas	TM	2019	*Triticum aestivum*	+	1.101	0.747
UFVPY367	Boa Esperança	Centro Sul	2019	*Triticum aestivum*	+	1.332	0.830
UFVPY375	Santa Juliana	TM	2019	*Triticum aestivum*	+	1.071	0.700
UFVPY376	Patrocínio	TM	2019	*Triticum aestivum*	+	1.157	0.629
UFVPY385	Uberaba	TM	2019	*Triticum aestivum*	+	1.163	0.449
UFVPY400	São Gotardo	TM	2019	*Triticum aestivum*	+	1.106	0.746
UFVPY450	Uberaba	TM	2019	*Panicum maximum*	+	1.192	0.667
UFVPY463	Uberaba	TM	2019	*Urochloa* sp.	-	1.246	0.572
UFVPY464	Patos de Minas	TM	2019	*Triticum aestivum*	+	1.031	0.629
UFVPY506	Santa Juliana	TM	2019	*Triticum aestivum*	+	1.069	0.629
UFVPY529	Santa Juliana	TM	2019	*Triticum aestivum*	+	0.811	0.491
UFVPY537	Patos de Minas	TM	2019	*Triticum aestivum*	+	0.948	0.355
UFVPY599	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	1.229	0.602
UFVPY604	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	1.272	0.624

UFVPY652	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.514	1.188
UFVPY656	Catas A. Noruega	Centro Sul	2019	*Urochloa* sp.	-	0.294	0.235
UFVPY661	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	1.037	0.567
UFVPY677	Campos Altos	TM	2019	*Urochloa* sp.	-	0.205	0.055
UFVPY733	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.829	0.506
UFVPY741	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.941	0.417
UFVPY742	Madre de Deus	Centro Sul	2019	*Urochloa* sp.	+	1.332	0.821
UFVPY758	Madre de Deus	Centro Sul	2019	*Urochloa* sp.	+	0.970	0.428
UFVPY801	Uberaba	TM	2019	*Triticum aestivum*	+	1.024	0.733
UFVPY813	Boa Esperança	Centro Sul	2019	*Triticum aestivum*	+	0.709	0.214
UFVPY815	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.927	0.613
UFVPY886	Ibiá	TM	2019	*Triticum aestivum*	+	1.011	0.584
UFVPY889	Patrocinio	TM	2019	*Triticum aestivum*	+	0.854	0.343
MOT	Passo Fundo	RS	2019	*Triticum aestivum*	+	0.654	0.287

S.2. Proportion of germination of PoT (C17 +) and non-PoT (C17 -) isolates obtained using the discriminatory dose (D.D.) in *in vitro* experiments for QoI fungicide group.

Isolate Code	City	Region	Year	Host	C17	Proportion of germination	
						AZOX D.D.= 5 µg/mL	PYRA D.D.= 1.3 µg/mL
UFVPY1	Uberaba	TM	2018	*Panicum maximum*	-	0.298	0.045
UFVPY21	Madre de Deus	Centro Sul	2018	*Urochloa* sp.	-	0.195	0.017
UFVPY70	Viçosa	Centro Sul	2018	*Triticum aestivum*	+	1.000	0.960
UFVPY92	Caxambú	Centro Sul	2018	*Urochloa* sp.	-	0.996	0.018
UFVPY108	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.042	0.014
UFVPY110	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.427	0.010
UFVPY112	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.515	0.033
UFVPY118	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.827	0.010
UFVPY119	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.044	0.040
UFVPY120	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.409	0.014
UFVPY121	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.517	0.013
UFVPY135	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.800	0.013
UFVPY158	Uberaba	TM	2018	*Urochloa* sp.	-	0.872	0.117
UFVPY165	Uberaba	TM	2018	*Triticum aestivum*	+	1.000	0.247
UFVPY166	Uberaba	TM	2018	*Urochloa* sp.	-	0.366	0.006
UFVPY167	Patos de Minas	TM	2018	*Triticum aestivum*	+	1.000	0.261
UFVPY168	Patos de Minas	TM	2018	*Triticum aestivum*	+	0.884	0.598
UFVPY171	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.268	0.014
UFVPY196	Campos Altos	TM	2019	*Urochloa* sp.	-	0.626	0.037
UFVPY197	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.483	0.139
UFVPY200	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.453	0.136
UFVPY203	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.111	0.011
UFVPY209	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.015	0.015
UFVPY210	Santana da Vargem	Centro Sul	2019	*Panicum maximum*	-	0.065	0.007

UFVPY213	S. Gonçalo do Pará	Centro Sul	2019	*Urochloa* sp.	+	0.960	0.830
UFVPY221	Ibiá	TM	2019	*Triticum aestivum*	+	1.000	0.331
UFVPY224	Ibiá	TM	2019	*Triticum aestivum*	+	0.959	0.395
UFVPY238	Ibiá	TM	2019	*Triticum aestivum*	+	0.939	0.054
UFVPY239	Ibiá	TM	2019	*Triticum aestivum*	+	0.908	0.023
UFVPY255	Santa Juliana	TM	2019	*Triticum aestivum*	+	0.959	0.200
UFVPY271	Campos Altos	TM	2019	*Triticum aestivum*	+	0.986	0.180
UFVPY276	Campos Altos	TM	2019	*Triticum aestivum*	+	0.990	0.653
UFVPY309	Boa Esperança	Centro Sul	2019	*Triticum aestivum*	+	1.000	0.017
UFVPY311	Boa Esperança	Centro Sul	2018	*Triticum aestivum*	+	0.993	0.516
UFVPY312	Boa Esperança	Centro Sul	2018	*Triticum aestivum*	+	0.993	0.847
UFVPY360	Patos de Minas	TM	2019	*Triticum aestivum*	+	0.986	0.555
UFVPY367	Boa Esperança	Centro Sul	2019	*Triticum aestivum*	+	0.996	0.910
UFVPY375	Santa Juliana	TM	2019	*Triticum aestivum*	+	0.982	0.768
UFVPY376	Patrocínio	TM	2019	*Triticum aestivum*	+	1.000	0.962
UFVPY385	Uberaba	TM	2019	*Triticum aestivum*	+	0.976	0.545
UFVPY400	São Gotardo	TM	2019	*Triticum aestivum*	+	0.973	0.187
UFVPY450	Uberaba	TM	2019	*Panicum maximum*	+	0.996	0.827
UFVPY463	Uberaba	TM	2019	*Urochloa* sp.	-	0.842	0.026
UFVPY464	Patos de Minas	TM	2019	*Triticum aestivum*	+	0.986	0.314
UFVPY506	Santa Juliana	TM	2019	*Triticum aestivum*	+	0.982	0.174
UFVPY529	Santa Juliana	TM	2019	*Triticum aestivum*	+	1.000	0.277
UFVPY537	Patos de Minas	TM	2019	*Triticum aestivum*	+	0.940	0.623
UFVPY599	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.959	0.735
UFVPY604	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.996	0.677
UFVPY652	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	1.000	0.796
UFVPY656	Catas A. Noruega	Centro Sul	2019	*Urochloa* sp.	-	0.743	0.132

UFVPY661	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.972	0.720
UFVPY677	Campos Altos	TM	2019	*Urochloa* sp.	-	0.560	0.210
UFVPY733	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.956	0.880
UFVPY741	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.983	0.604
UFVPY742	Madre de Deus	Centro Sul	2019	*Urochloa* sp.	+	0.983	0.481
UFVPY758	Madre de Deus	Centro Sul	2019	*Urochloa* sp.	+	0.800	0.661
UFVPY801	Uberaba	TM	2019	*Triticum aestivum*	+	0.986	0.361
UFVPY813	Boa Esperança	Centro Sul	2019	*Triticum aestivum*	+	0.993	0.763
UFVPY815	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	1.000	0.667
UFVPY886	Ibiá	TM	2019	*Triticum aestivum*	+	0.986	0.433
UFVPY889	Patrocinio	TM	2019	*Triticum aestivum*	+	0.976	0.174

S.3. Proportion of germination of PoT (C17 +) and non-PoT (C17 -) isolates obtained using the discriminatory dose (D.D.) in *in vitro* experiments for SDHI fungicide group.

Isolate Code	City	Region	Year	Host	C17	Proportion of germination		
						BENZ D.D.= 0.005 µg/mL	FLUX D.D.= 13.5 µg/mL	BIXA D.D.= 4 µg/mL
UFVPY1	Uberaba	TM	2018	*Panicum maximum*	-	0.045	0.087	0.023
UFVPY21	Madre de Deus	Centro Sul	2018	*Urochloa* sp.	-	0.028	0.014	0.055
UFVPY70	Viçosa	Centro Sul	2018	*Triticum aestivum*	+	1.000	0.830	0.024
UFVPY92	Caxambú	Centro Sul	2018	*Urochloa* sp.	-	0.197	0.914	0.108
UFVPY108	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.462	0.077	0.040
UFVPY110	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.164	0.010	0.01
UFVPY112	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.387	0.558	0.006
UFVPY118	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.420	0.559	0.017
UFVPY119	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.309	0.199	0.010
UFVPY120	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.021	0.039	0.019
UFVPY121	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.031	0.010	0.040
UFVPY135	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.016	0.003	0.012
UFVPY158	Uberaba	TM	2018	*Urochloa* sp.	-	1.000	0.356	0.013
UFVPY165	Uberaba	TM	2018	*Triticum aestivum*	+	0.905	0.888	0.125
UFVPY166	Uberaba	TM	2018	*Urochloa* sp.	-	0.561	0.082	0.040
UFVPY167	Patos de Minas	TM	2018	*Triticum aestivum*	+	0.003	0.221	0.033
UFVPY168	Patos de Minas	TM	2018	*Triticum aestivum*	+	0.906	0.375	0.041
UFVPY171	Patos de Minas	TM	2018	*Urochloa* sp.	-	0.285	0.082	0.228
UFVPY196	Campos Altos	TM	2019	*Urochloa* sp.	-	0.232	0.174	0.090
UFVPY197	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.516	0.113	0.023
UFVPY200	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.343	0.026	0.037
UFVPY203	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.134	0.011	0.011
UFVPY209	Formiga	Centro Sul	2019	*Urochloa* sp.	-	0.078	0.572	0.013
UFVPY210	S. da	Centro Sul	2019	*Panicum*	-	0.335	0.015	0.038

	Vargem			*maximum*				
UFVPY213	S. Gonçalo Pará	Centro Sul	2019	*Urochloa* sp.	+	0.033	0.013	0.263
UFVPY221	Ibiá	TM	2019	*Triticum aestivum*	+	0.870	0.873	0.459
UFVPY224	Ibiá	TM	2019	*Triticum aestivum*	+	0.681	0.174	0.066
UFVPY238	Ibiá	TM	2019	*Triticum aestivum*	+	0.706	0.266	0.332
UFVPY239	Ibiá	TM	2019	*Triticum aestivum*	+	0.705	0.315	0.043
UFVPY255	Santa Juliana	TM	2019	*Triticum aestivum*	+	0.404	0.034	0.376
UFVPY271	Campos Altos	TM	2019	*Triticum aestivum*	+	0.899	0.795	0.013
UFVPY276	Campos Altos	TM	2019	*Triticum aestivum*	+	0.710	0.050	0.111
UFVPY309	Boa Esperança	Centro Sul	2019	*Triticum aestivum*	+	0.881	0.615	0.221
UFVPY311	Boa Esperança	Centro Sul	2018	*Triticum aestivum*	+	0.706	0.155	0.096
UFVPY312	Boa Esperança	Centro Sul	2018	*Triticum aestivum*	+	0.802	0.773	0.165
UFVPY360	Patos de Minas	TM	2019	*Triticum aestivum*	+	0.613	0.911	0.264
UFVPY367	Boa Esperança	Centro Sul	2019	*Triticum aestivum*	+	0.875	0.855	0.090
UFVPY375	Santa Juliana	TM	2019	*Triticum aestivum*	+	0.949	0.983	0.024
UFVPY376	Patrocínio	TM	2019	*Triticum aestivum*	+	0.809	0.989	0.074
UFVPY385	Uberaba	TM	2019	*Triticum aestivum*	+	0.896	0.819	0.042
UFVPY400	São Gotardo	TM	2019	*Triticum aestivum*	+	0.725	0.923	0.010
UFVPY450	Uberaba	TM	2019	*Panicum maximum*	+	0.942	0.895	0.112
UFVPY463	Uberaba	TM	2019	*Urochloa* sp.	-	0.769	0.775	0.032
UFVPY464	Patos de Minas	TM	2019	*Triticum aestivum*	+	0.785	0.715	0.036
UFVPY506	Santa Juliana	TM	2019	*Triticum aestivum*	+	0.866	0.784	0.191
UFVPY529	Santa Juliana	TM	2019	*Triticum aestivum*	+	0.996	0.706	0.093
UFVPY537	Patos de Minas	TM	2019	*Triticum aestivum*	+	0.893	0.923	0.150
UFVPY599	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.906	0.856	0.514
UFVPY604	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.973	0.869	0.384
UFVPY652	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.922	0.837	0.263

UFVPY656	Catas A. Noruega	Centro Sul	2019	*Urochloa* sp.	-	1.000	0.238	0.007
UFVPY661	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.674	0.800	0.266
UFVPY677	Campos Altos	TM	2019	*Urochloa* sp.	-	0.840	0.378	0.018
UFVPY733	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.933	0.766	0.685
UFVPY741	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.949	0.892	0.140
UFVPY742	Madre de Deus	Centro Sul	2019	*Urochloa* sp.	+	0.989	0.916	0.557
UFVPY758	Madre de Deus	Centro Sul	2019	*Urochloa* sp.	+	0.945	0.155	0.565
UFVPY801	Uberaba	TM	2019	*Triticum aestivum*	+	0.832	0.842	0.060
UFVPY813	Boa Esperança	Centro Sul	2019	*Triticum aestivum*	+	0.945	0.844	0.294
UFVPY815	Madre de Deus	Centro Sul	2019	*Triticum aestivum*	+	0.854	0.881	0.016
UFVPY886	Ibiá	TM	2019	*Triticum aestivum*	+	0.749	0.779	0.055
UFVPY889	Patrocinio	TM	2019	*Triticum aestivum*	+	0.416	0.983	0.306