FRANKLIN JACKSON MACHADO

IS *Fusarium meridionale* MORE ADAPTED AS GIBBERELLA EAR AND STALK ROT PATHOGEN OF MAIZE THAN *F. graminearum*? COMPARATIVE EPIDEMIOLOGY AND TOXIGENICITY

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Plant Pathology at the College of Agriculture, Food and Environment of University of Kentucky, United States of America, with co-tutelage with the Graduate Program of the Federal University of Viçosa, Brazil.

Adviser: Emerson Medeiros Del Ponte

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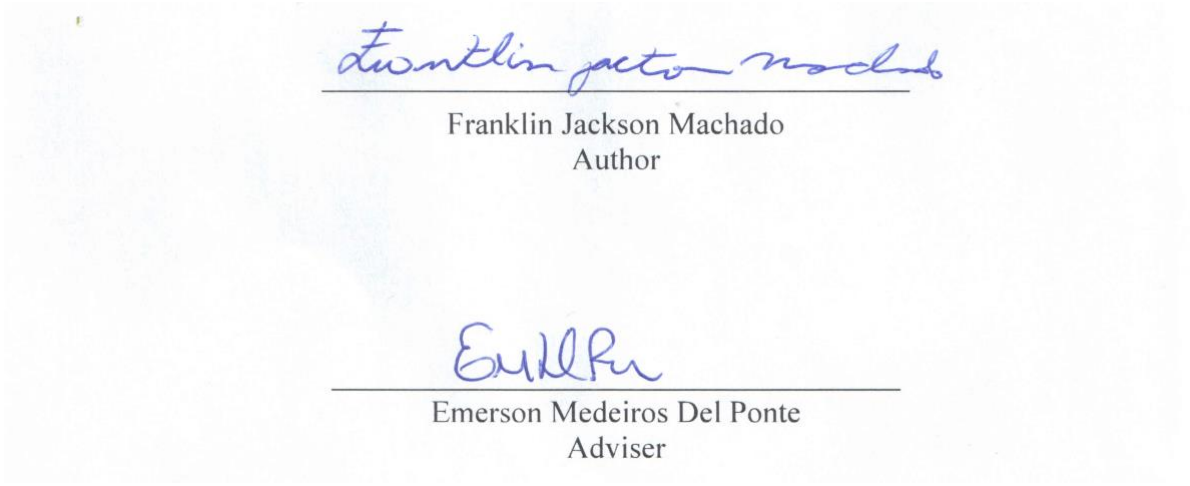

Franklin Jackson Machado
Author

Emerson Medeiros Del Ponte
Adviser

Aos meus amados pais, Lena e Olegário

Às minhas queridas irmãs, Flávia e Cláudia

Aos meus lindos sobrinhos, Daniel e Arthur

À minha companheira de todas as horas, Aline

Aos meus familiares e amigos que sempre torceram por mim

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BIOGRAPHY

FRANKLIN JACKSON MACHADO, son of Lenilda de Oliveira Xavier Machado and Olegário de Souza Machado, was born in Salinas, Minas Gerais, June 23, 1991. In 2014 he was awarded B.S. in Agronomy from the Universidade Federal de Viçosa, Brazil. In 2016 he was awarded MS degree in Plant Pathology from the same institution and in the same year, he started his Doctorate also mentored by Dr. Emerson M. Del Ponte. In 2018, he joined the Department of Plant Pathology to pursue PhD degree working in the lab of Dr. Lisa J. Vaillancourt at the University of Kentucky as the first student enrolled in the Dual Degree program of UFV and UK.

RESUMO

MACHADO, Franklin Jackson, D.Sc., Universidade Federal de Viçosa, dezembro de 2019. ***Fusarium meridionale* é mais adaptado como patógeno da Podridão de espiga e de colmo do milho que *F. graminearum*? epidemiologia comparativa e toxigenicidade.** Orientador: Emerson Medeiros Del Ponte.

Podridão de Gibberella em espiga (GER) e em colmo (GSR) do milho no Brasil são causadas principalmente por *Fusarium meridionale*, uma espécie pertencente ao complexo de espécies de *Fusarium graminearum* (FGSC). Outra espécie do complexo, *F. graminearum*, é a causa mais importante da giberela do trigo e a segunda em milho no Brasil e a principal ou a única causa de GER e GSR em muitos países. Uma hipótese para essa mudança observada na dominância em diferentes hospedeiros, onde estas espécies coocorrem, é que *F. meridionale* é mais adaptado ao milho que *F. graminearum* e vice-versa. Realizamos uma análise comparativa de características patogênicas e saprofíticas para uma coleção de isolados representando as duas espécies e tendo como hospedeiros de origem o trigo ou o milho, a fim de testar esta hipótese. Primeiramente, foi realizado um estudo de campo em quatro locais usando três diferentes híbridos comerciais de milho para comparar a agressividade (medida da severidade de GER) de dois isolados de *F. meridionale* e dois de *F. graminearum*, todos obtidos do milho, inoculadas sozinhos ou sequencialmente alternados no estágio de florescimento. No geral, a severidade de GER foi maior nas plantas inoculadas apenas com *F. meridionale* (52,1%); intermediário quando a inoculação com *F. meridionale* foi seguida por *F. graminearum* (Fmer→Fgr, 40,3%) ou quando *F. graminearum* foi seguida por *F. meridionale* (Fgra→Fmer, 38,3%); e menor nas plantas inoculadas apenas com *F. graminearum* (23,8%). Somente a micotoxina nivalenol (NIV) foi detectada em grãos das inoculações com *F. meridionale*, e desoxinivalenol (DON) foi a única micotoxina encontrada em inoculações com *F. graminearum*. Quantidades aproximadamente iguais de NIV e DON (1,2: 1), juntamente com zearalenona (ZON), foram encontradas no tratamento Fmer→Fgr, mas apenas NIV pôde ser detectada nos tratamentos Fgra→Fmer. Estes resultados sugerem que *F. meridionale* é mais agressivo ao milho que *F. graminearum*. Entretanto, este experimento incluiu apenas um pequeno número de isolados e, em um segundo estudo, usamos uma amostra muito maior composta por 16 isolados de *F. graminearum* (12 de trigo, quatro de milho) e 25 isolados de *F. meridionale* (nove de trigo, 16 de milho) para inocular espigas e colmos de milho no campo. Foram realizados ensaios de campo com quatro híbridos comerciais de milho durante o inverno e o verão. O hospedeiro de origem não fez diferença na severidade de GER ou GSR causada por isolados de ambas as espécies. Entretanto, isolados de *F. meridionale* foram duas vezes mais

agressivos que isolados de *F. graminearum* em espigas, enquanto *F. graminearum* foi, em média, um pouco mais agressivo em colmos de milho que *F. meridionale*, independentemente do híbrido. Cerca de metade dos isolados de *F. graminearum* produziam principalmente DON e 15ADON, enquanto dois terços dos isolados de *F. meridionale* produziam apenas NIV. Não foram detectadas micotoxinas nas amostras restantes. Em um terceiro estudo, uma coleção pouco maior consistindo de 18 isolados de *F. graminearum* (12 de trigo e seis de milho) e 27 isolados de *F. meridionale* (nove de trigo e 18 de milho) foram comparados em relação a 17 diferentes características saprofíticas e patogênicas e toxigênicas. Embora tenha existido variação intra-específica significativa para a maioria das características, os isolados foram fortemente estruturados por espécie, independentemente do hospedeiro de origem, com base na análise multivariada. Os resultados mostraram que *F. graminearum* era um patógeno mais agressivo ao trigo e produziu maior número de macroconídios, peritécios e ascósporos em meio de cultura. Todos os isolados de *F. graminearum* produziram principalmente DON em culturas de arroz ou em espigas de trigo. Por outro lado, *F. meridionale* foi um colonizador mais agressivo de estigmas de milho e cresceu mais rapidamente em meio de cultura. Todos os isolados de *F. meridionale* produziram principalmente NIV *in vitro* e *in planta*, com exceção de dois isolados de milho que produziram mais DON em espigas de trigo. Os resultados deste estudo contribuem com novos dados sobre a biologia dessas espécies em condições subtropicais e tropicais no Brasil. Eles sugerem uma agressividade e toxigenicidade diferencial como explicações parciais para a predominância de *F. meridionale* no milho e *F. graminearum* no trigo, estabelecendo bases para o desenvolvimento de novas hipóteses para explicar essas associações.

Palavras-chave: *Triticum aestivum*. *Zea mays*. Giberela do trigo. Nivalenol. Desoxinivalenol.

ABSTRACT

MACHADO, Franklin Jackson, D.Sc., Universidade Federal de Viçosa, December, 2019. **Is *Fusarium meridionale* more adapted as Gibberella Ear and Stalk Rot pathogen of maize than *F. graminearum*? comparative epidemiology and toxigenicity.** Adviser: Emerson M. Del Ponte.

Gibberella ear (GER) and stalk rot (GSR) diseases of maize in Brazil are caused mainly by *Fusarium meridionale*, a species belonging to the *Fusarium graminearum* species complex (FGSC). Another species within this complex, *F. graminearum*, is second in importance on maize but is the main species causing Fusarium Head Blight disease of wheat in Brazil. This species is also the predominant cause of GER and GSR in North America, where *F. meridionale* has never been found. One hypothesis for the observed shift in dominance on different hosts where these species co-occur is that *F. meridionale* is more fit and adapted on maize than *F. graminearum*, and vice-versa. We undertook a thorough comparative analysis of pathogenic and saprophytic fitness-related traits using a collection of strains representative of the two species and hosts of origin in order to test this hypothesis. First, four field trials were conducted at different locations and hybrids to compare the aggressiveness (measure of GER severity) of two *F. meridionale* and two *F. graminearum* strains, all isolated from maize, inoculated singly or sequentially and alternately at the silking stage. Overall, GER severity was highest in plants inoculated with *F. meridionale* alone (52.1%); intermediate when inoculation with *F. meridionale* was followed by *F. graminearum* (Fmer→Fgra, 40.3%) or when *F. graminearum* was followed by *F. meridionale* (Fgra→Fmer, 38.3%); and lowest in plants inoculated with *F. graminearum* alone (23.8%). Only nivalenol (NIV) mycotoxin was detected in kernels from *F. meridionale* inoculations, and deoxynivalenol (DON) was the only mycotoxin found in *F. graminearum* inoculations. Approximately equal amounts of NIV and DON (1.2:1), along with zearalenone (ZON), were found in Fmer→Fgra, but only NIV could be detected in the Fgra→Fmer treatments. These results suggested that *F. meridionale* was more aggressive to maize than *F. graminearum*. However, this experiment included only a small number of isolates and so in a second study we used a much larger sample comprised of 16 isolates of *F. graminearum* (12 from wheat, four from maize), and 25 isolates of *F. meridionale* (nine from wheat, 16 from maize) to inoculate maize ears and stalks in the field. Field trials with four commercial maize hybrids were conducted during the winter and the summer growing seasons in Viçosa, MG. The host of origin made no difference in GER or GSR severity caused by isolates of either species. However, *F. meridionale* isolates were twice as aggressive as *F. graminearum* isolates infecting ears, while *F. graminearum* was, on average, slightly more aggressive on maize stalks than *F. meridionale*, regardless of the hybrid. Around half of *F. graminearum* strains

produced primarily DON and 15ADON, whereas two thirds of *F. meridionale* strains produced only NIV. Mycotoxins were not detected in the remaining samples. In a third study, a slightly expanded collection consisting of 18 *F. graminearum* isolates (12 from wheat and six from maize) and 27 *F. meridionale* isolates (nine from wheat and 18 from maize) were compared in relation to 17 different saprophytic, pathogenic, and toxigenic traits. Although there was significant intraspecies variation for most traits, the strains were strongly structured by species regardless of the host of origin, based on a multivariate analysis. Results showed that *F. graminearum* was a more aggressive pathogen of wheat and produced higher numbers of macroconidia, perithecia, and ascospores in culture. All *F. graminearum* strains produced primarily DON in rice cultures or in wheat heads. On the other hand, *F. meridionale* was a more aggressive colonizer of maize silks and grew faster in culture. All *F. meridionale* strains produced mainly NIV both *in vitro* and *in planta*, with the exception of two strains from maize that produced more DON in wheat heads. The results of this study contribute new data on the biology of these species in subtropical and tropical conditions in Brazil. They suggest that differential aggressiveness and toxigenicity as partial explanations for the predominance of *F. meridionale* on maize and *F. graminearum* on wheat, and they lay a foundation for developing further hypotheses to explain these associations.

Keywords: *Triticum aestivum*. *Zea mays*. Fusarium head blight. Nivalenol. Deoxynivalenol.

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GENERAL INTRODUCTION

Fusarium head blight (FHB) and Gibberella ear and stalk rot (GER and GSR) are fungal diseases of major impact to wheat and maize, respectively, worldwide. These diseases cause significant economic losses due to reduction in grain yield, but also reduce crop value due to contamination of grain with mycotoxins that are regulated for maximum tolerance limits.

In Brazil, a handful of species within the *Fusarium graminearum* species complex (FGSC) cause these three diseases, but the dominant species vary according to the host. In maize, *F. meridionale*, is found most often causing GER and GSR, and it is also recovered most frequently from perithecia produced on maize stubble. On the other hand, FHB in wheat and barley is caused mainly by *F. graminearum*. Among the toxins produced by FGSC, B-trichothecenes and zearalenone are produced in larger quantities. The B-trichothecenes are deoxynivalenol (DON) and nivalenol (NIV) (and respective acetyls 3-ADON, 15-ADON and 4-ANIV). While *F. graminearum* have either deoxynivalenol DON or NIV, depending on the region, *F. meridionale* is a consistent NIV-producing species. An individual strain can be assigned to a chemotype based on the B-trichothecene and acetylate produced in the highest amount. The most common chemotypes are 3-ADON or 15-ADON (strains produce DON and smaller amount of 3-ADON or 15-ADON), or NIV. *Fusarium graminearum* strains affecting cereals in Brazil are only of the 15-ADON chemotype.

Although more than one member of the FGSC can co-occur in the same field or even in the same plant or plant part, the effect of interspecific interactions on disease development is largely unknown, especially under Brazilian conditions. The role of saprophytic fitness on disease development is also poorly understood. Host preference was suggested by surveys of regions where multiple FGSC species and cereal hosts occur, in which skewed host-species associations

were observed. However, few studies have been published and little is known about the existence of host preferences among members of the FGSC. Previous studies with a small number of Brazilian strains of *F. graminearum* and *F. meridionale* from wheat showed that they were similarly aggressive towards wheat. However, due to the small sample size, this could be an isolate- rather than species-specific trait. This dissertation explores the hypothesis that the relative dominance in Brazil of *F. meridionale* on maize and of *F. graminearum* on wheat is due to differential abilities as a saprophyte, and/or as a pathogen on maize versus wheat. We conducted a thorough comparison of pathogenic and saprophytic fitness-related traits for a large number of strains representative of the two species and hosts of origin, in order to enhance our knowledge of the host preference mechanism.

This dissertation is structured as a series of three studies. The first two were based on field experiments conducted under tropical and subtropical conditions in Brazil, each including at least two consecutive growing seasons. In these studies, we tested our hypothesis that *F. meridionale* is more aggressive on maize ears and stalks than *F. graminearum* when inoculated singly, or sequentially alternated in co-inoculations in the field conditions. We included isolates of both species collected from wheat and from maize to investigate whether host origin had any influence on relative aggressiveness. We measured for the first time trichothecene and ZON levels in grains harvested from inoculated maize ears. In a third study, conducted during my Dual Degree period at the University of Kentucky, USA, a multivariate analysis was performed based on characterization of a variety of phenotypes *in vitro* and *in vivo* that are relevant to saprophytic, pathogenic, and toxigenic ability. This work provides a basis for a better understanding of the role of host vs. pathogen factors in shaping FGSC populations infecting wheat and maize in Brazil.

ARTICLE 1

Gibberella ear rot in maize ears caused by *Fusarium meridionale* and *F. graminearum*: a single vs. sequential alternating species inoculation

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ABSTRACT

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In Brazil, a handful of species within the *Fusarium graminearum* species complex (FGSC) infect cereal crops at relative frequencies that vary according to the host. In maize, *F. meridionale* is the most prevalent FGSC species causing both ear and stalk rots (GER and GSR) as well as producing perithecial inoculum on maize stubble. In contrast, another species in the FGSC, *F. graminearum*, is the most common species causing Fusarium head blight (FHB) in wheat in the same region. The cause of this difference in dominance is unknown but may be related to relative degrees of host adaptation between the two species. A four location-hybrid study was conducted to compare the aggressiveness (measure of GER severity) of *F. meridionale* (Fmer) and *F. graminearum* (Fgra) strains, both isolated from maize. These were inoculated singly or sequentially alternated at the silking stage, totaling four treatments: Fgra and Fmer (each inoculated alone, four days after silk emergence), and Fgra→Fmer and Fmer→Fgra (sequentially inoculated, six days apart). The mean GER severity was highest in Fmer (52.1%), intermediate in Fmer→Fgra (40.3%) and Fgra→Fmer (38.3%) and lowest in Fgra (23.8%). The production of mycotoxins deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZON) was assessed in one experiment. Only NIV was detected in kernels after inoculation of Fmer alone, and DON was the only toxin found after inoculation of Fgra. Both NIV and DON (1.2:1 ratio), together with ZON, were found in grains harvested from the Fmer→Fgra sequential treatment. In contrast, only NIV was found in the Fgra→Fmer treatment. Our results suggest that *F. meridionale* is more aggressive to maize ears than *F.*

graminearum. Our results contribute new knowledge to the epidemiology of GER in Brazil and confirm the need to focus attention on the presence of NIV in maize grains, which is neglected in the current regulatory legislation.

Key words: *Zea mays*, *Fusarium graminearum* species complex, nivalenol, interaction, FGSC.

INTRODUCTION

Several species within the *Fusarium graminearum* species complex (FGSC) are the cause of major diseases of winter and summer cereal crops, including Fusarium head blight (FHB) in wheat (McMullen et al. 2012) and Gibberella ear rot (GER) and stalk rot (GSR) in maize (Goswami and Kistler 2004; Munkvold 2003a). FGSC members are particularly common as cereal pathogens in the subtropical climate of southern Brazil where wheat is grown during the winter-spring and maize during the summer-fall seasons. GER is favored by increased levels of moisture around silking, followed by moderate temperatures and high rainfall during the maturation period (Munkvold 2003a; Sutton 1982). Above-normal rainfall during El Nino years in the south of Brazil, and an increasing use of irrigation, has increased GER risk. As a result, trichothecene mycotoxins typically produced by GER pathogens have been found contaminating commercial maize grain (Oliveira et al. 2017).

Both FHB in wheat and barley and GER of maize are known to reduce yield (Duffeck et al. 2019; Munkvold 2003a) but the presence of mycotoxins that accumulate in the kernels and reduce product value is of increasing concern to producers (Munkvold et al. 2019). FGSC species are known to produce several mycotoxins, but the most important ones belong to the B-trichothecene group, including nivalenol (NIV) and deoxynivalenol (DON) (Miller and Greenhalgh 1991). These mycotoxins can accumulate in grain at levels considered unsafe for both livestock and human consumption, posing a serious threat to food safety (Pestka 2010; Rocha et

al. 2005). To mitigate the impact on animal and human health, maximum tolerated limits have been established for *Fusarium* mycotoxins in wheat and maize grain and byproducts in Brazil and worldwide (ANVISA, 2011; van Egmond et al. 2007). In Brazil, DON, ZON and fumonisins (B1 and B2) are the only mycotoxins that are currently regulated for maize (ANVISA, 2011, 2017).

Host shifts in dominance among different FGSC members have been reported in South America (Castañares et al. 2016; Del Ponte et al. 2015; Gomes et al. 2015, 201; Kuhnem et al. 2016; Sampietro et al. 2011). For example, *F. graminearum* of the 15ADON genotype is the most prevalent species causing FHB in wheat and barley in South America and worldwide (Del Ponte et al. 2015; Kelly and Ward 2018). In Brazil, *F. meridionale*, a NIV-producing species, is the dominant FGSC species in maize, and also is increasing in importance in wheat in regions where maize is a major crop (Del Ponte et al. 2015). A few other NIV-producing FGSC species have been found in association with GER in maize in other regions of the world, usually at minor frequency, including *F. graminearum*, *F. asiaticum*, *F. cortaderiae* and *F. austroamericanum*, and also some non-FGSC species (*F. culmorum*, *F. cerealis* and *F. poae*) (Basler 2016; Desjardins and Proctor 2011; Kuhnem et al. 2016; Lee et al. 2012; Ndoye et al. 2012).

A recent survey of mycotoxins contaminating maize grains in southern Brazil was consistent with our previous work suggesting that *F. meridionale* was the primary maize pathogen. The survey found that NIV mycotoxin, which is produced mainly by *F. meridionale*, and rarely by *F. graminearum*, was present in more samples (76%) than the DON mycotoxin (48%), though levels for both were lower than the Brazilian limits of contamination for DON (Oliveira et al. 2017).

Management of GER aims to reduce infection by toxigenic fungi in order to suppress mycotoxin production (Munkvold 2003). The most effective control for GER (and also Fusarium ear rot - FER) is the use of host genetic resistance. However, breeding for resistance to GER and FER is complicated by the diversity of pathogenic species (Mesterházy et al. 2012; Munkvold

2003). Complete resistance to GER has not been reported and the mechanisms underlying resistance are not completely understood (Mesterházy et al. 2012). In fact, when each of the 14-most planted maize hybrids in southern Brazil was challenged with *F. meridionale*, none was resistant to GER (Nerbass et al. 2015). The use of foliar fungicides to control maize diseases (Esker et al. 2018), including ear rots, has increased in recent years (Andriolli et al. 2016; Anderson et al. 2017; Luna and Wise 2015; Fingstag et al. 2019).

In North America, GER is caused mainly by *F. graminearum* of the 15ADON genotype although the 3ADON genotype can also be an important contributor depending on the region (Burlakoti et al. 2017; Kuhnem et al. 2015). The toxin profile of a *Fusarium* species has been considered to influence pathogenesis (Ward et al. 2002). While DON has been confirmed as an aggressiveness factor that facilitates fungus spread within wheat heads and maize ears in several studies (Bai et al. 2002; Desjardins et al. 1996; Harris et al. 1999; Maier et al. 2006), the role of NIV in the infection and spread of the disease within the maize ear remains unclear (Maier et al. 2006). There are very few studies that investigate differential pathogenicity among members of the FGSC with different toxin profiles that cause GER. In South Africa, *F. boothii*, a 15ADON-producing species that was dominant on maize (Boutigny et al. 2011), produced more severe symptoms and higher levels of mycotoxins compared with DON-producing *F. graminearum* isolates that were highly aggressive to wheat (Beukes et al. 2018, 2). In that study, only the *F. boothii* isolates were obtained from maize, while the *F. graminearum* isolates had been recovered from wheat. Multiple members of the FGSC and of other species complexes can co-occur in the same field or even in the same maize ears (Logrieco et al. 2002; Oldenburg et al. 2017; Picot et al. 2012), but the effects of any interspecific interaction on disease development are unknown. In this study we tested the hypothesis that *F. meridionale* from maize is more aggressive to maize ears, and produces significant levels of NIV trichothecenes, when compared with *F. graminearum* also

obtained from maize. Isolates were inoculated singly, and they were also co-inoculated sequentially and alternately in order to study any interspecific interactions.

MATERIAL AND METHODS

Fungal isolates and inoculum preparation

Two *F. graminearum* (DON-producing) isolates and two *F. meridionale* (NIV-producing) isolates were selected from a collection obtained from symptomatic maize kernels (Stumpf et al. 2013). These isolates had been identified to species as part of a previous study that showed the dominance of *F. meridionale* in Brazilian maize (Kuhnem et al. 2016).

A spore suspension was prepared by growing isolates individually on potato dextrose agar (PDA) for 10 days with a 12-h dark/light cycle. The macroconidia were filtered through two layers of cheesecloth to reduce the number of mycelial fragments present in the inoculum. The macroconidial suspensions of each isolate were quantified by using a haemocytometer. Spore suspensions of the two isolates of each species were diluted in sterile water and then mixed in a 1:1 ratio (v/v) to achieve the desired macroconidia concentrations (5×10^5 macroconidia/ml) (Reid et al. 1995).

Field trial in Southern Brazil (subtropics)

Field experiments were carried out in a no-till area located in the municipality of Eldorado do Sul, Rio Grande do Sul (RS) state, during the 2012 and 2013 growing seasons, and in the municipality of Lages, Santa Catarina (SC) state, during the 2012-growing season. The locations are ~ 400 km apart. In each location and year, two field trials, sown on different dates, were arranged in a 2x5 factorial experiment in a split-plot design with four replications. Two ear-rot-susceptible commercial maize hybrids, P30F53 HR® and STATUS TL®, were randomly assigned to the main plots, each of which consisted of five rows (5-m-long row with 60 cm between rows). The subplot

units consisted of five plots of one row each, for which five inoculation treatments were randomized. The ten central plants of each row were inoculated. All plots were bordered by uninoculated rows of the same maize hybrid.

Field trials in Southeastern Brazil (Tropics)

One field experiment was conducted at the experimental station at the Universidade Federal de Viçosa (20º44'44" S, 42º50'59" W, 661 m above sea level) during the 2017 winter growing season. Seeds of the maize hybrid RB9004 PRO2® were sown in April that year. The field trials were arranged in a randomized complete block design with four replications, and each of the treatments was randomly assigned to the experimental units (Table 1). Each block consisted of an individual row. Each plot consisted of a 5-m-long row with 60 cm between rows. The central ten plants in each plot were inoculated. All plots were bordered by uninoculated rows of the same maize hybrid. Plots were fertilized following chemical soil analyses, and sprinkle-irrigated as needed. Weather variables (precipitation - rain plus irrigation; maximum, average and minimum relative humidity - RH; and maximum, average and minimum temperature) were recorded hourly by an automatic meteorological station (Squitter, Squitter Soluções em Monitoramento Ambiental, São José dos Campos, São Paulo, Brazil). The station was located within the field and data were collected from sowing to maize harvest. Average weather variables are presented in Figure S2.

Inoculation procedures

The five inoculation treatments are summarized in Table 1. Single inoculation treatments were made at four days after silking (Reid et al. 2002). Plants were individually inoculated by injecting the suspensions (2 ml of a $5 \times 10_5$ macroconidia/ml solution) into the silk channel of the primary ear using a syringe with an obtuse needle (Anderson et al. 2016; Reid et al. 1995). In order to determine whether there was a positive or negative interaction of the two species in co-

inoculations, sequential, alternated inoculations were made six days apart. A mock inoculation (sterile distilled water) was included as a negative control. Inoculations were followed by three consecutive days of irrigation. When grain moisture content reached an estimated 22%, the ten inoculated ears for each treatment were handpicked, husked, and the GER severity score on each ear was rated as the percentage area of the ear that was symptomatic (Reid et al. 2002).

Mycotoxin analysis

Mycotoxins were measured for the field experiment conducted in Viçosa in 2017. Harvested maize ears were dried at 60 °C for five consecutive days, shelled, and grains were stored dry at -20 °C until analysis. Mycotoxin in grains was determined by bulking the individual ears from each block, which was considered as a replicate. A 10-g sub-sample of each pooled replicate was ground by using a coffee grinder and then homogenized. The ground samples were sent to the Virginia Tech Deoxynivalenol (DON) Testing Lab. The amount of DON and each of its acetylated forms (15ADON and 3ADON), NIV, and ZON were quantified using a gas chromatography–mass spectrometry method as described previously (Fuentes et al. 2005; Mirocha et al. 1998).

Table 1. Single and sequential inoculation treatments of *Fusarium graminearum* (Fgra) and *Fusarium meridionale* (Fmer) on maize ears.

Treatment[a]	**Silking + 4 days**	**Silking + 10 days**
Fgra	*F. graminearum*	-
Fmer	*F. meridionale*	-
Fgra →Fmer	*F. graminearum*	*F. meridionale*
Fmer→Fgra	*F. meridionale*	*F. graminearum*
Mock inoculations	Water	Water

[a] All inoculations were done by injecting 2 mL of the macroconidia suspension at 5×10^5 macroconidia/ml. All isolates were obtained from naturally infected maize kernels (Stumpf et al. 2013).
[b]Mix of two *F. graminearum* (15ADON) isolates.
[c]Mix of two *F. meridionale* (NIV) isolates.

Data analysis

The main and interaction effects of the treatment factors on the response variable were evaluated under a linear mixed modelling framework at 5% significance. Trials, maize hybrids, and replicates were treated as random effects in our model. The model was expanded to account for the main and interaction effects of the species from each host of origin. The mixed models were fitted using the *lmer* function of 'lme4' package (Bates et al. 2015) of R (R Core Team 2019). The 'emmeans' package (Lenth 2019) was used to estimate the back-transformed lsmeans and respective confidence intervals. The function *cld* from 'multcomp' R package (Hothorn et al. 2008) was used for multiple comparison of treatment means at 5% significance.

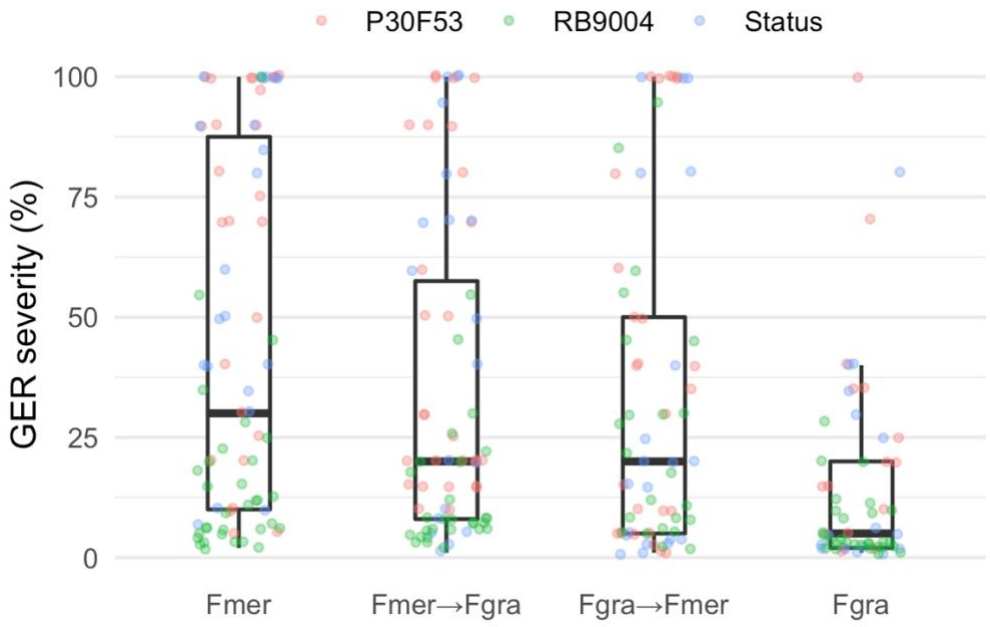

Figure 1. Distribution of Gibberella ear rot (GER) severity among the inoculation treatments of *Fusarium graminearum* (Fgra) and *F. meridionale* (Fgra) isolates on three different hybrids (P30F53 HR®, STATUS TL® and RB9004 PRO2®) in three different locations in Brazil. The first two maize hybrids were planted during 2012 growing season in Lages Santa Catarina state (SC) and Eldorado do Sul, Rio Grande do Sul state (RS), and during 2013 growing season in Eldorado do Sul, RS. The RB9004 PRO2® maize hybrid was cultivated during 2017 growing season in Viçosa, Minas Gerais state (MG). The line within each box represents the median, the top and bottom lines of the boxes represent the 75th and 25th percentiles, respectively. The vertical bars extending beyond the boxes is show the 10th and 90th percentiles, and the dots represent the GER severity of each inoculated ear.

RESULTS

Mean GER severity differed among the treatments ($P < 0.001$); it ranged from 1.00% to 100% (median = 20%) being lowest and highest in Fgra and Fmer, respectively, compared with the other treatments ($P < 0.05$) (Fig. 1). Severity induced by Fmer (52.1%) was two times higher than by Fgra (23.8%) (Fig. 2). Severity in Fgra→Fmer and Fmer→Fgra treatments did not differ from one another ($P = 0.793$) but differed from each of the single inoculations (Table 2). No visible GER symptoms were found in the uninoculated controls of any experiment, suggesting no influence of background inoculum.

Table 2. Estimated differences in mean Gibberella ear rot (GER) severity between pairs of inoculum treatments of *Fusarium graminearum* (Fgra) and *F. meridionale* (Fmer) isolates on three different hybrids (P30F53 HR®, STATUS TL® and RB9004 PRO2®) in three different field locations in Brazil during 2012, 2013 and 2017 growing seasons in Lages-SC, Eldorado do Sul-RS and Viçosa-MG, Brazil.

Contrasts	Estimate	SE[a]	df[b]	t.ratio	P-value[c]
Fgra - (Fgra→Fmer)	-14.50	4.87	247	-2.97	0.017
Fgra - Fmer	-28.30	4.66	242	-6.07	< 0.001
Fgra - (Fmer→Fgra)	-16.50	4.75	244	-3.47	0.004
(Fgra→Fmer) - Fmer	-13.80	4.54	246	-3.05	0.0134
(Fgra→Fmer) - (Fmer→Fgra)	-2.00	4.55	240	-0.44	0.972
Fmer - (Fmer→Fgra)	11.80	4.41	239	2.69	0.038

[a]SE = Standard error.
[b]Degrees-of-freedom method: Kenward-Roger.
[c]*P*-value adjustment: Tukey method for comparing a family of 4 estimates.

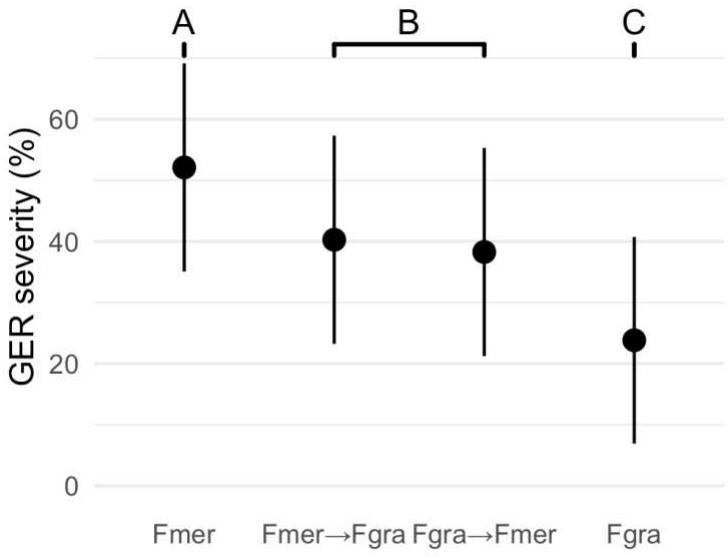

Figure 2. Least square means and confidence intervals from linear mixed analyses of the effect of single and sequential inoculation treatments of *Fusarium graminearum* (Fgra) and *F. meridionale* (Fgra) isolates on Gibberella ear rot (GER) severity of inoculated maize ears of three different hybrids (P30F53 HR®, STATUS TL® and RB9004 PRO2®) in three different locations in Brazil. Means with the same letters are not significantly different from each other based on Tukey test (*P* = 0.05).

Trichothecene levels in kernels from Fmer and Fgra treatments ranged from 0.50 to 2.05 µg/g (ppm) respectively (Fig. 2B). For the sequential inoculation treatments, DON and NIV levels ranged from 0.35 to 3.15 µg/g in the Fgra→Fmer treatment. DON was detected in only one sample of Fmer→Fgra at similar levels with NIV and ZON (0.70 µg/g, 0.60 µg/g and 0.40 µg/g, respectively). Only NIV was detected in kernels from ears inoculated with Fmer, whereas DON was the only mycotoxin detected in kernels from the Fgra treatment. In contrast, NIV was more abundant in kernels from ears of the Fgra→Fmer treatment. Finally, in the Fmer→Fgra treatment NIV, DON, and a smaller amount of ZON were detected (Fig. 3A). Neither 15ADON nor 3ADON were detected in any sample (Fig. 3A). There was a positive correlation between NIV production and GER severity (Fig. 3B).

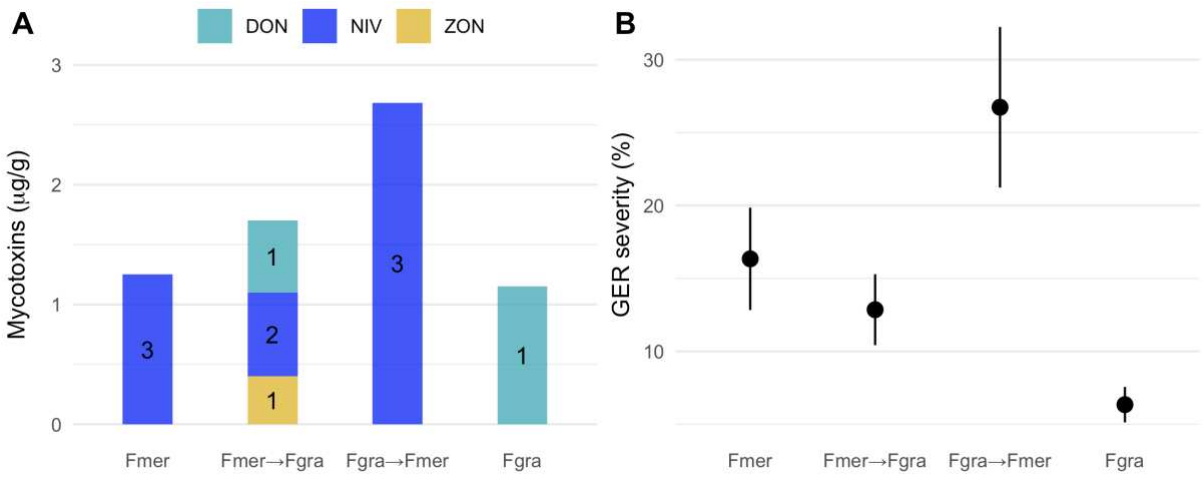

Fig. 3. (A) Mean production of deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZON) and **(B)** mean Gibberella ear rot (GER) severity and standard error resulting from *Fusarium graminearum* and *F. meridionale* isolates inoculated onto maize ears of hybrid RB9004 PRO2® in single and sequential inoculation treatments in a field trial conducted in Viçosa 2017. Numbers represent how many of the four blocks (bulked sample of kernels from 10 inoculated ears) had detectable mycotoxins. Measurements of 15-acetyl-deoxynivalenol (15ADON) and 3ADON, and in the missing blocks, were under the limit of detection (< 0.25 µg/g).

DISCUSSION

In this study we have tested the hypothesis that *F. meridionale* from maize is more aggressive to maize ears than maize isolates of *F. graminearum*. Evidence of potential host preference among

members of the FGSC has been reported from different parts of the world where multiple FGSC species infect different cereal crops (Boutigny et al. 2011; Carter et al. 2000; Del Ponte et al. 2015; Umpiérrez-Failache et al. 2013; van der Lee et al. 2015). For example, differential aggressiveness among FGSC species inoculated in the same host has been reported for FHB in wheat (Goswami and Kistler 2005; Nicolli et al. 2015; Spolti et al. 2012; Tóth et al. 2005). In contrast, colonization did not differ between *F. meridionale* and *F. graminearum* inoculated onto soybean pods in the field (Chiotta et al. 2016). In most of the previous studies of GER, *F. boothii*, the dominant species associated with the disease in South Africa and China, produced the same amount of disease as *F. graminearum* in some field experiments (Beukes et al. 2018; Gai et al. 2017). One study from China reported that *F. boothii* from maize was similarly aggressive to maize stalks as *F. graminearum* isolates obtained from maize or wheat (Zhang et al. 2016). In our study, we found that severity was more than twice as high when *F. meridionale* was inoculated singly compared with *F. graminearum*. This supports our hypothesis that *F. meridionale* is more aggressive to maize, which may contribute to its dominance in naturally infected ears and stalks of maize in a region where *F. graminearum* is dominant in wheat - and thus its inoculum is not limited and is also found in maize (Kuhnem et al. 2016).

The current work is the first field inoculation study to confirm the ability of *F. meridionale* to produce significant amounts of NIV in maize ears. This mycotoxin was recently found to be the most frequent mycotoxin in surveys of commercial maize grain in Brazil (Oliveira et al. 2017), which supports our hypothesis of the importance of this pathogen to GER epidemics. The higher toxin amount/severity ratio for *F. graminearum* than *F. meridionale* that we observed may be due to the generally high toxigenic potential of *F. graminearum* of the DON type versus the NIV-producing FGSC (Nicolli et al. 2015; Tóth et al. 2005). In fact, *F. graminearum* produced 17 times more DON *in vitro* in rice cultures than *F. meridionale* isolates produced NIV (Chapters 2 and 3).

During sequential co-inoculations, GER severity and NIV production by *F. meridionale* were both reduced if *F. graminearum* was inoculated following *F. meridionale* at the same infection site. It seems that *F. meridionale* facilitated *F. graminearum* infection but compromised its own development as a result. Previous studies of maize ears inoculated with a mix of species suggested that *F. graminearum* predisposed ear tissues to infection by a weaker species, *F. verticillioides*, in mixed inoculation treatment (Reid et al. 1999). The damage caused by the first species to be inoculated may have an effect similar to wounding caused by lepidopteran larvae that facilitates colonization by subsequently applied inoculum (Picot et al. 2012). However, when *F. graminearum* was inoculated first in our experiments, its growth was apparently ultimately suppressed by the more aggressive *F. meridionale*, evidenced by absence of DON in these samples. Additional experiments including mycotoxin analyses and fungal biomass measurements are needed to confirm and extend these findings.

The large amount of variation in pathogenicity between strains of the same species, limited sample sizes, and a focus on isolated components of the disease cycle (e.g. colonization) limits our ability to conclude that there is a role of host preference in shaping FGSC composition in the field. Nonetheless, the collective results of our extensive surveys of species composition frequency and of these field experiments suggest that relative aggressiveness is likely to be one of the drivers shaping FGSC composition across major cereals in Brazil. Future studies should focus on increasing sample size from each species and investigating other components of the disease cycle such as asexual and sexual reproduction ability.

This study contributes new knowledge about the aggressive and competitive behavior of two co-occurring FGSC species that cause GER in maize in Brazil. Our findings are important to breeders because they can inform the selection of appropriate adapted species to be used as inoculum when screening for host resistance. Ideally, further studies should focus on screening of regionally-dominant inoculum where the hybrids shall be deployed, as well as testing the

significance of the species x hybrid interactions using larger number of host genotypes, similar to what has been done for wheat genotypes challenged with *F. graminearum* and *F. meridionale* (Mendes et al. 2018).

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SUPPLEMENTARY MATERIAL

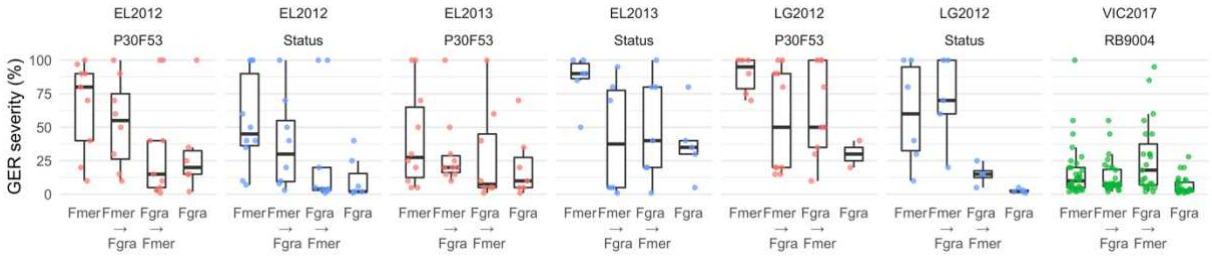

Fig S1. Distribution of Gibberella ear rot (GER) severity among the inoculation treatments of *Fusarium graminearum* (Fgra) and *F. meridionale* (Fgra) isolates on three different hybrids (P30F53 HR®, STATUS TL® and RB9004 PRO2®) in three different locations in Brazil by trial. The first two maize hybrids were planted during 2012 growing season in Lages (LG), Santa Catarina state (SC) and Eldorado do Sul (EL), Rio Grande do Sul state (RS), and during 2013 growing season in Eldorado do Sul, RS. The RB9004 PRO2® maize hybrid was cultivated during 2017 growing season in Viçosa (VIC), Minas Gerais state (MG). Each data point represents the GER severity in a single ear.

Figure S2. Daily average of meteorological data recorded by an automatic meteorological station located within the field trial conducted in Viçosa, Minas Gerais state (MG) during the 2017 growing season. Minimum and maximum air temperature (°C) are represented in red and blue solid lines, respectively. Precipitation (rain plus irrigation) is represented by vertical bars. The RB9004 PRO2® maize hybrid was planted on April 25, inoculated on the 7th of August and harvested on the 25th of September.

ARTICLE 2

Gibberella ear and stalk rot caused by *Fusarium meridionale* and *F. graminearum*: aggressiveness and mycotoxin production

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ABSTRACT

Machado, F. J., Arruda, R., Schmale III, D. G., Vaillancourt, L. J., Del Ponte, E. M. 2020. Gibberella ear and stalk rot caused by *Fusarium meridionale* and *F. graminearum*: aggressiveness and mycotoxin production. *XXXXX*: xx-xx.

Gibberella ear (GER) and stalk rot (GSR) of maize in Brazil are caused mainly by *Fusarium meridionale*, while *F. graminearum*, the dominant pathogen causing Fusarium head blight (FHB) in wheat worldwide, is the main contributor to FHB epidemics in the same regions. One hypothesis for this observed shift in dominance is that *F. meridionale* is more adapted as a maize pathogen, while *F. graminearum* is more adapted as a wheat pathogen. We tested a collection consisting of 16 isolates of *F. graminearum* (12 from wheat and four from maize) and 24 isolates of *F. meridionale* (8 from wheat and 16 from maize) for aggressiveness (on ears and stalks) and toxin production in kernels from field inoculations of four maize hybrids. Field trials were conducted during the winter and the summer growing seasons. Ear and stalk inoculations were performed four days after silking. Inoculated maize ears and stalks were harvested at R5-R6 and disease severity was estimated. The amount of deoxynivalenol (DON) and its acetylated forms (15ADON and 3ADON), nivalenol (NIV) and zearalenone (ZON) were quantified in harvested grains from one season (summer). Average GER severity induced by *F. meridionale* (13.94%) was twice as high as *F. graminearum* (7.15%). Host of origin (wheat versus maize) had no significant effect on GER severity. There was large variability in GER and GSR severity among the isolates of the same species. The GER and GSR severity varied from 0.33 to 100% and from 0.005 to 81.17%, respectively. On average, *F. graminearum* (18.40%) was slightly more aggressive than *F. meridionale* (16.10%), regardless of the hybrid and host of origin. The primary mycotoxins produced by *F. graminearum* were DON and 15ADON (7/16 strains), and NIV was the only toxin produced by *F. meridionale* (17/24 strains). Three isolates of each species produced ZON. The rest of the strains did not produce detectable mycotoxins. Our results provide a basis for

understanding the epidemiology of both species in Brazil, contributing new knowledge to explain the predominance of *F. meridionale* associated with maize. Further studies focusing on other components of the disease cycle, such as saprophyte behavior and reproductive ability, may help to further understand the nature of the host dominance patterns we have observed.

Key-words: *Zea mays*, nivalenol, deoxynivalenol, comparative epidemiology.

INTRODUCTION

Members of the *Fusarium graminearum* species complex (FGSC) are ascomycete fungi that cause Fusarium head blight (FHB) in small grains, and Gibberella ear rot (GER) and stalk rot (GSR) on maize. These diseases cause significant reductions in the yield and grain quality worldwide (Kazan et al. 2012; McMullen et al. 2012). They also pose a threat to food safety because of the ability of the causal fungi to produce mycotoxins that accumulate in the kernels at unsafe levels for both livestock and human ingestion (Rocha et al. 2005; Pestka 2010). The primary toxins produced by FGSC are the B-trichothecenes, which include deoxynivalenol (DON) and nivalenol (NIV) (and their acetylated forms), as well as zearalenone (ZON) (Miller and Greenhalgh 1991). DON and ZON are proposed to act as aggressiveness factors in maize stalks (Quesada-Ocampo et al. 2016), and the importance of DON for aggressiveness has been well-established on wheat heads and maize ears (Bai et al. 2002; Desjardins et al. 1996; Harris et al. 1999; Maier et al. 2006). The role of NIV during maize ear and stalk infection is less well understood (Maier et al. 2006).

In Brazil, GER and GSR are more prevalent in the southern subtropical climates where small grains are typically cultivated in a no-tillage system, and FHB is of common occurrence (Del Ponte et al. 2009). Ear and stalk rots caused by FGSC are increasing in importance as the application of improved technologies, and agronomic practices including irrigation and double-

cropping have expanded (Costa et al. 2019). The recent promulgation of mycotoxin limits for maize-based food and feed produced in Brazil (ANVISA 2011), and the unpredictability of fungicide performance for disease control (Andriolli et al. 2016), means that breeding for host resistance is a priority (Mesterházy et al. 2012). Resistance to either GSR or GER in maize is a complicated trait that is influenced by genetic background and environmental factors, as well as by the pathogen population (Mesterházy et al. 2012; Yang et al. 2010). To date, there are no reports of complete resistance to GER or GSR, and the mechanisms of quantitative resistance are not completely understood (Mesterházy et al. 2012). As we discussed in Chapter 1, breeders should rely on adapted strains of regionally dominant species when screening for host resistance.

The hypothesis that FGSC pathogen species vary in their relative levels of adaptation to different crops is suggested by the results of surveys in regions where multiple FGSC species and cereal hosts occur (Carter et al. 2000; Boutigny et al. 2011; Del Ponte et al. 2015; Kuhnem et al. 2016; Umpiérrez-Failache et al. 2013). There are some controlled studies that also support this hypothesis: for example, the most prevalent species causing FHB, *F. graminearum* 15ADON, has been shown to be more fit as a pathogen of wheat compared with other species (Liu et al. 2017; Nicolli et al. 2018; Zhang et al. 2016). Likewise, in the previous chapter, *F. meridionale* was more aggressive and competitive than *F. graminearum* when it was used to inoculate or co-inoculate maize ears. However, the small sample size in the study in Chapter 1 (two isolates of each species), the combining of the isolates, thus masking any intraspecific differences, and the focus on only one stage of the cycle (colonization) and one single organ (ears), limit our ability to conclude that this relative fitness has a major role in shaping FGSC composition in the field. In the current study, the number of isolates was increased, isolates were collected from both maize and wheat, isolates were evaluated individually, and their ability to colonize stalks was also studied. The main objective of our work was to compare *F. graminearum* and *F. meridionale* aggressiveness on maize ears and stalks, and mycotoxin production in ears.

MATERIALS AND METHODS

Collection of isolates

Forty-one isolates of *F. graminearum* and *F. meridionale* that were collected during previous surveys from symptomatic maize (Kuhnem et al. 2016) and wheat kernels (Del Ponte et al. 2015) were used in the trials. These isolates were previously identified simultaneously to species and trichothecene genotype (Del Ponte et al. 2015, Kuhnem et al. 2016). The isolates were recovered from storage and their identities were confirmed by using the Fg16F/R primer set (Nicholson et al. 1998) that yields distinct fragment sizes for *F. meridionale* (~500 bp) or *F. graminearum* (~450 bp) (Astolfi et al. 2011; Castañares et al. 2016; Del Ponte et al. 2015; Nicholson et al. 1998). The 40 isolates were selected to be representative geographically, and for year of sampling. The number of isolates from each host and each species was based on the reported frequencies in surveys. Thus a total of 25 *F. meridionale* (nine from wheat and 16 from maize) and 16 *F. graminearum* (12 from wheat and four from maize) were selected. The isolates are described in Table S1.

Inoculum preparation

Spore suspensions were prepared by growing the isolates on *Spezieller Nahrstoffarmer* agar (SNA) plates for 10 days at 23 °C with a 12-h dark/light cycle. The macroconidia were filtered through two layers of cheesecloth to reduce the mycelial fragments present in the inoculum. The macroconidial suspension was then quantified by using a hemocytometer. Sterile water was added to the inoculum to achieve the desired macroconidial concentration (2×10^5 macroconidia/ml) (Reid et al. 2002; Kuhnem et al. 2015; Anderson et al. 2016; Chungu et al. 1996).

Field experiments

Field experiments were conducted at the experimental station at the Universidade Federal de Viçosa (20º44'44" S, 42º50'59" W, 661 m above sea level) during two growing seasons consisting of a winter crop in 2017 and a summer crop in 2018. In each growing season, two field trials were sown (60 cm between rows) on different dates three weeks apart and with different commercial maize hybrids. During the 2017 growing season, SupremoViptera® (Syngenta) and RB9004 PRO2® (KWS sementes) hybrids were sown between April and May. The hybrids MG580PW® (Dow AgroSciences) and BM820® (Sementes Biomatrix) were planted between October and early-November during the 2018 growing season. Two trials were planted side-by-side, one for the GER assay and the other for the GSR assay. Plants were fertilized following chemical soil analyses, and sprinkle-irrigated as needed.

The experiment was laid out in a randomized complete block design with four replications. Each block was an individual row. The isolates were randomly assigned to the plot (experimental units consisted of a 1-m-long row). The central three plants in each plot were inoculated. All plots were bordered by uninoculated rows of the same maize hybrid.

Inoculation procedures

GER trials. Plants were inoculated by injecting 2 ml of each spore suspension into the silk channel of the primary ear of each plant four days after silk emergence by using a syringe with an 5 cm long and 3 mm diameter obtuse needle (Reid et al. 2002) (Fig. 1A). Ears mock-inoculated with sterile distilled water served as negative controls. The plots were irrigated for two consecutive days after inoculation. When plants reached the R5-R6 stage (dent to physiological maturity), ears were handpicked and husked (Fig. 1B). GER severity was scored visually as the percent symptomatic area of each ear (Reid and Zhu 2005).

GSR trials. Maize stalks were inoculated four days after silk emergence. One ml of each inoculum suspension (2×10^5 macroconidia/ml) was injected at a 45° angle downward into the middle of the first internode above the uppermost aerial root of each plant by using a syringe with an obtuse needle (Reid and Zhu 2005) (Fig. 1C). The obtuse needle was 5 cm long and 3 mm diameter with an opening on the underside near the tip (Reid and Zhu 2005). A mock inoculation (sterile distilled water) was included as a negative control. Plants were irrigated for two consecutive days after inoculation. At the R5-R6 stage (dent to physiological maturity), stalks were split longitudinally and symptomatic internodes were photographed (Fig. 1D). The percent diseased (discolored) area of the internode (Reid and Zhu 2005) was measured by using Assess software (Lamari 2002).

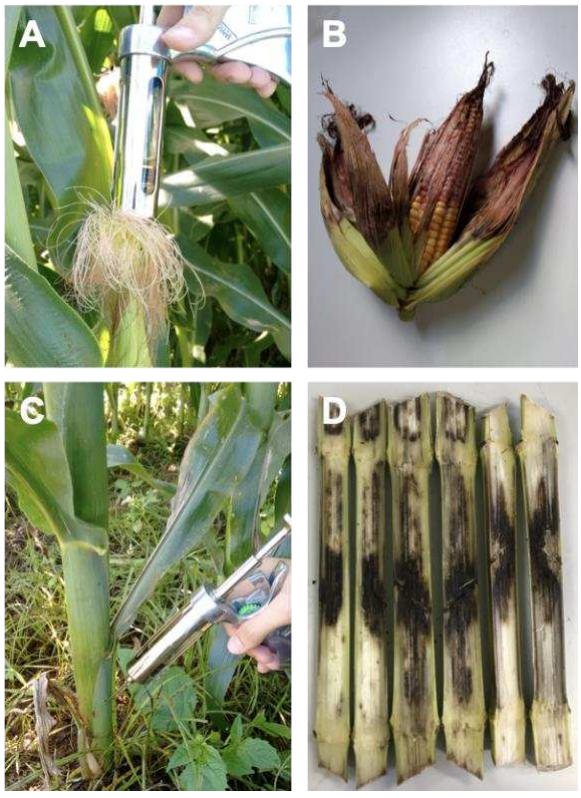

Figure 1. Procedure used to inoculate maize ears (A) and stalks (C) with *Fusarium graminearum* and *F. meridionale* isolates, and symptoms of Gibberella ear (B) and stalk (D) rot at dent to physiological maturity stage.

Meteorological data

Weather variables including precipitation (P, rain and irrigation), temperature (T, maximum and minimum), and relative humidity (RH), were recorded hourly at the experimental site by using an automatic meteorological station (Squitter, Squitter Soluções em Monitoramento Ambiental, São José dos Campos, São Paulo, Brazil). The station was located within the field and data were collected from sowing to maize harvest. Daily values of the weather variables are presented in Figure S1.

Mycotoxin analysis

Mycotoxins were analyzed in maize hybrid BM820® kernels harvested from the experiment conducted in 2018. Harvested ears were dried on a greenhouse bench for five days, shelled, and grains were kept at -20 °C until analysis. Mycotoxin was determined by bulking grains of all individual ears inoculated with the same isolate. A 10-g sub-sample of each pooled replicate was ground by using a coffee grinder and then homogenized. The amount of DON and its acetylated forms (15ADON and 3ADON), NIV, and ZON were quantified using a gas chromatography–mass spectrometry method as described previously (Fuentes et al. 2005; Mirocha et al. 1998).

Data analysis

The effects of the main factors (species and host of origin), and the interaction between them, were evaluated in a multilevel nested mixed model framework. Maize hybrids, isolates and replicates were treated as random effects. GSR and GER severity data (on a percentage scale) were log-transformed (logSEV = log[SEV + 1]) to stabilize variances. The mixed model analysis was performed using the lmer function of package 'lmer4' (Bates et al. 2015) of R (R Core Team 2019). The effects were tested at a 5% significance level. The 'emmeans' package (Lenth 2019) was used to estimate the least square means and respective confidence intervals. The function 'cld' from R

package ‘multcomp’ (Hothorn et al. 2008) was used for comparing the treatment means at 5% significance.

RESULTS

Gibberella ear rot

All isolates were pathogenic to maize ears, but there was a large degree of variation in symptom severity (Fig. 2). Mean severity differed between the species, but was dependent on the host of origin, as suggested by the significant interaction term (P = 0.034). The severity of disease was similar for isolates from wheat and from maize for *F.graminearum* (P = 0.079) and for *F. meridionale* isolates (P = 0.25). However, when comparing isolates obtained only from maize, the severity was twice as high in ears inoculated with *F. meridionale* versus *F. graminearum* (P = 0.002). On average, the GER severity on ears inoculated with *F. graminearum* and *F. meridionale* from maize was 7.15% (± 5.30 standard error [SE]) and 13.94% (± 9.41 SE), respectively (Fig. 4A). In contrast, there was no difference in severity between the two species when isolates were obtained only from wheat (P = 0.464). In this case, the average GER severity was 10.58% (± 7.31 SE) and 11.84% (± 8.14 SE) for *F. graminearum* and *F. meridionale*, respectively (Fig. 4A).

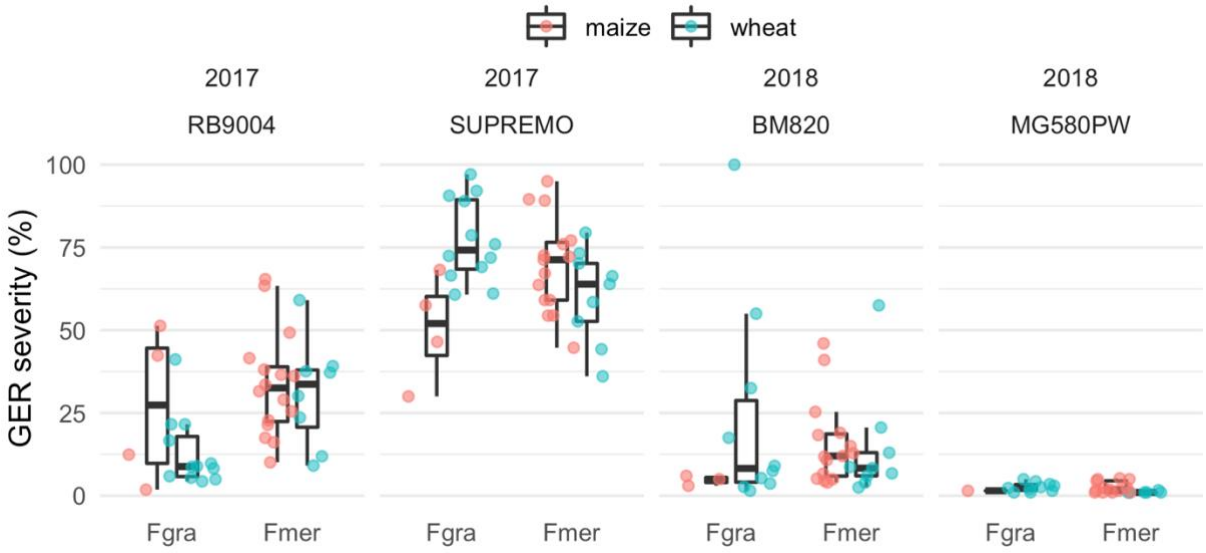

Figure 2. Severity of Gibberella ear rot on four different commercial maize hybrids (Supremo Viptera®, RB9004 PRO2®, MG580PW®, BM820®) inoculated with 16 isolates of *F. graminearum* (Fgra) or 24 isolates of *F. meridionale* (Fmer). Data points for the isolates obtained from infected maize kernels are shown as red dots, and from symptomatic wheat heads as blue dots. The first two maize hybrids were planted during the 2017 growing season, and the last two were cultivated during 2018 growing season. The line within the box represents the median, whereas the top and bottom lines of the boxes represent the 75th and 25th percentiles of the data, respectively. The vertical bars extending beyond the boxes represent 10th and 90th percentiles, and the dots represent the mean GER severity for each isolate across all inoculated ears.

Gibberella stalk rot

Similar to GER, all isolates were pathogenic to the stalks. Mean GSR severity varied among the isolates ($P < 0.001$), ranging from trace levels to 81.17% (median = 18.50%) (Fig. 3). The interaction tested in the mixed model (species *vs* host) did not affect the GSR severity ($P = 0.168$). There was no significant difference between isolates from maize versus wheat ($P = 0.659$). However, there was a significant effect of the species, regardless of the hybrid or the host from which the isolates had been obtained ($P = 0.021$). On average, *F. graminearum* was slightly more aggressive on maize stalks (18.40% ± 7.06 SE) than *F. meridionale* (16.10% ± 6.19 SE) (Fig. 4B). There was no significant correlation between stalk and ear disease severity ($\rho = -0.10$; $P = 0.218$).

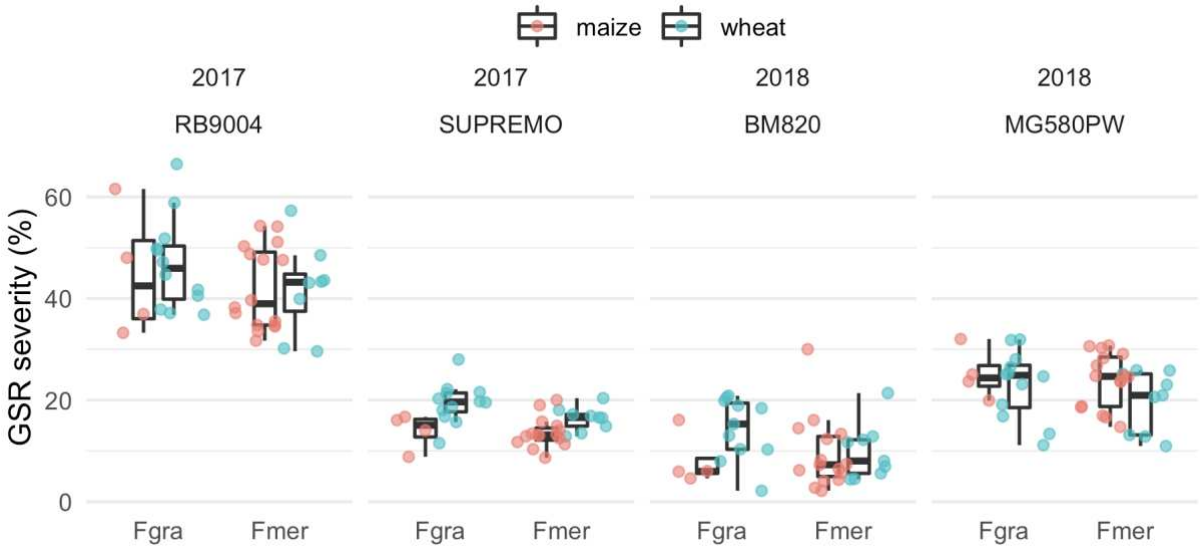

Figure 3. Severity of Gibberella stalk rot on four different commercial maize hybrids (Supremo Viptera®, RB9004 PRO2®, MG580PW®, BM820®) inoculated with 16 isolates of *F. graminearum* (Fgra) or 24 isolates of *F. meridionale* (Fmer). Data points for the isolates obtained from infected maize kernels are shown as red dots, and from symptomatic wheat heads as blue dots. The first two maize hybrids were planted during the 2017 growing season and the last two were cultivated during 2018 growing season. The line within the box represents the median, whereas the top and bottom lines of the boxes represent the 75th and 25th percentiles of the data, respectively. The vertical bars extending beyond the boxes represent 10th and 90th percentiles, and the dots represent the mean GSR severity for each isolate across all inoculated stalks.

Mycotoxin analysis

Not all isolates produced trichothecenes at detectable levels in the composite samples of kernels from all replicates. A larger proportion of *F. meridionale* isolates (17/24) produced detectable trichothecenes compared to *F. graminearum* (7/16). ZON was not detected in kernels from any of the ears inoculated with *F. meridionale*. This mycotoxin was found only in samples of kernels inoculated with three *F. graminearum* strains from maize, and three from wheat (Fig. 5).

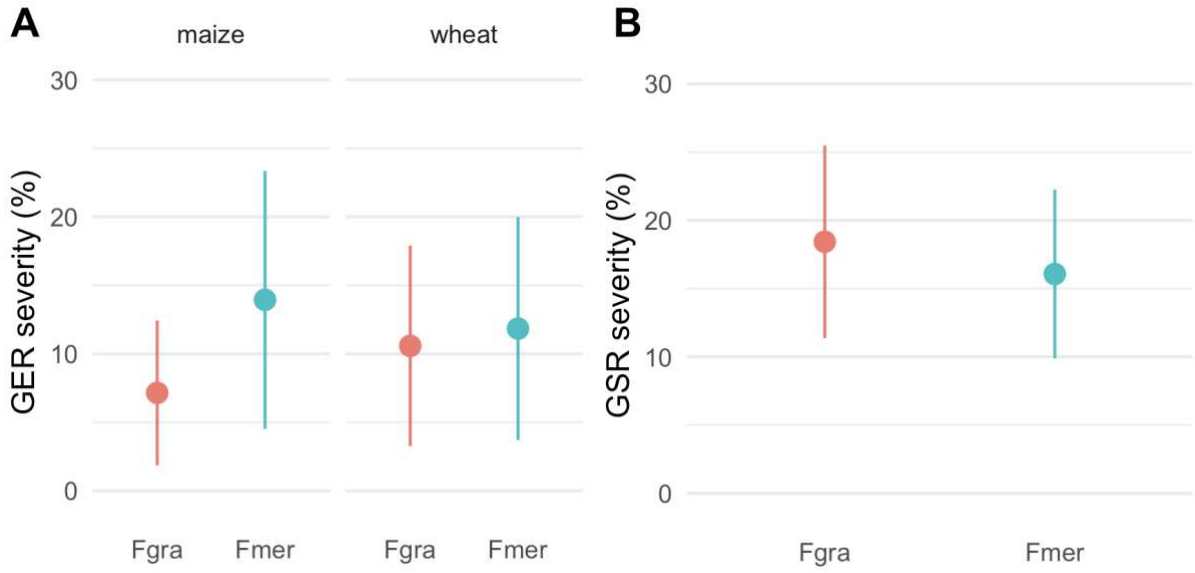

Figure 4. Least square means and standard error from linear mixed analyses of the effect of 16 isolates of *F. graminearum* (Fgra) or 24 isolates of *F. meridionale* (Fmer) obtained from either wheat or maize on (**A**) Gibberella ear rot (GER) and (**B**) Gibberella stalk rot (GSR) severity of inoculated maize ears and stalks across four different commercial maize hybrids (RB9004 PRO2®, SUPREMO®, BM820® and MG580PW®) in two growing seasons in Brazil. The first two maize hybrids were planted during 2017 growing season and the last two were cultivated during 2018 growing season.

NIV was detected only in kernels from ears inoculated with *F. meridionale,* while DON/15ADON were only detected in ears inoculated with *F. graminearum*. One sample of kernels from ears inoculated with a *F. graminearum* strain from wheat had levels of 3ADON that were equivalent to 15ADON. This strain produced the highest amount of DON overall (DON = 52.15 µg/g) and also produced ZON (Fig. 5). None of the other *F. graminearum* strains produced detectable 3ADON. On average, *F. graminearum* strains from wheat produced twice as much DON (13.36 µg/g) as those from maize (6.45 µg/g). In kernels from ears inoculated with *F. meridionale*, NIV levels ranged from 0.25 to 7.00 µg/g across the strains. Isolates from maize produced slightly more NIV (1.80 µg/g) than isolates from wheat (1.35 µg/g).

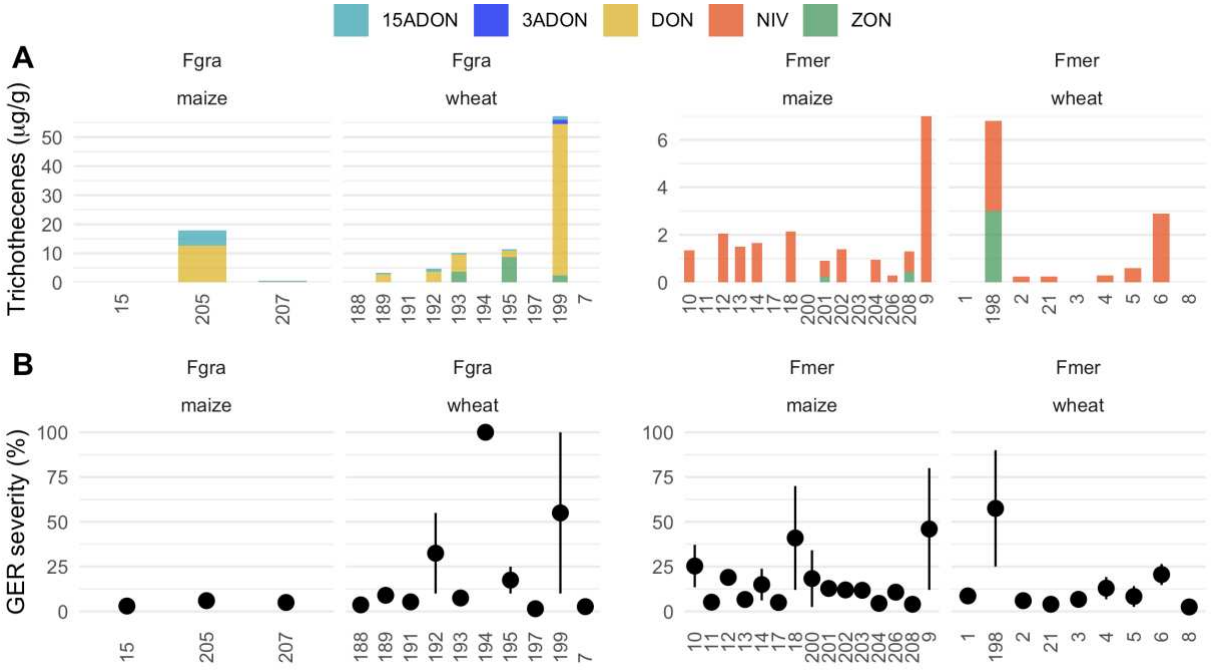

Fig. 5. (A) Mean production of deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZON) and **(B)** mean Gibberella ear rot (GER) severity (%) and standard error of *Fusarium graminearum* and *F. meridionale* isolates inoculated onto maize ears of hybrid BM820® in a field trial conducted in Viçosa during the 2018 summer season. Values represents a bulked sample of kernels from 5-6 inoculated ears. Limit of detection (< 0.25 µg/g).

There was a significant correlation between GER severity and DON production by *F. graminearum* isolates (ρ = 0.81; P = 0.027). For *F. meridionale* isolates, a significant positive correlation was also observed between GER severity and NIV production (ρ = 0.79; P < 0.01) (Fig. 5).

DISCUSSION

The presence of multiple *Fusarium* spp. in association with maize ear or stalk symptoms is well-known, and the diversity of species varies across regions (Kelly et al. 2017; Logrieco et al. 2002; Oldenburg et al. 2017; Picot et al. 2012). In the past, variation in species composition in different regions were most often attributed to prevailing weather conditions. Changes or variability in climatic patterns (Vaughan et al. 2016) or introduction of highly aggressive species (Ward et al.

2008) have been implicated as important drivers of rapid changes in relative dominance of species among pathogenic populations. The effect of the host on FGSC composition has emerged more recently with our increased ability to identify FGSC in larger collections of strains from multiple hosts in the same region (Del Ponte et al. 2015; Gomes et al. 2015; Kuhnem et al. 2016). Survey and experimental data (controlled environment and field studies) from Brazil have provided support for the hypothesis that *F. meridionale* is more adapted as a maize pathogen, while *F. graminearum* is more adapted as a wheat pathogen in regions where these species and crops co-occur. In this field study, pathogenicity traits on both ears and stalks, as well as toxigenicity traits, partially support the hypothesis that selection for relative aggressiveness of *F. meridionale* to maize ears plays a role in this adaptation, given that *F. meridionale* strains caused twice as much GER as *F. graminearum*, when those strains originated from maize.

The NIV mycotoxin was recently reported for the first time in Brazil, and was more frequently found than DON in surveys of commercial maize grain (Oliveira et al. 2017). Alarmingly, NIV is about 10 times more toxic to animals than DON (Desjardins 2008). We confirmed our results from Chapter 1, that *F. meridionale* isolates causing GER produce NIV in maize ears. *F. graminearum*-inoculated ears contained DON and no NIV, but levels of DON were seven times higher. Our results support the need to expand surveys of NIV in Brazil, and further revise the legislation which currently considers only DON and ZON (ANVISA 2011). Both NIV and DON were correlated with GER severity. There was also a relationship between mycotoxin production and host of origin, with isolates of *F. graminearum* from wheat producing more DON than those from maize, and isolates of *F. meridionale* from maize producing more NIV than the strains from wheat. This implies that there is differential selection for increased mycotoxin levels by these hosts and suggests that NIV plays a more important role in colonization of maize versus wheat, while higher levels of DON are more important for wheat colonization. The role of NIV mycotoxin in pathogenesis on maize should be further explored. Not all of our strains produced

detectable levels of mycotoxins in the composite sample of kernels from field inoculations. The significance of the correlation between severity on ears and toxin amount suggests that low levels of mycotoxin were likely undetected in those cases, due to technical limitations of the sampling and detection methods. These isolates should be further examined under controlled conditions *in vitro*. We confirmed the ability of the genotyping to predict the chemotype (Desjardins 2008; Ward et al. 2008): thus, DON and 15ADON were produced by *F. graminearum* isolates previously identified as the 15ADON genotype, while *F. meridionale* strains previously identified as the NIV genotype produced only NIV mycotoxins.

Previous surveys in southern Brazil showed that *F. meridionale* was much more abundant among isolates from perithecia formed on corn stubble in wheat fields, as well as from maize plants with stalk rot symptoms (Kuhnem et al. 2016). Its relatively high frequency on maize tissues in comparison with *F. graminearum* suggested a higher ability to colonize maize stalks, but this was not confirmed by our data when stalks were inoculated with either species under controlled conditions. Intriguingly, we found that *F. graminearum* was actually more aggressive on stalks than *F. meridionale*, although the difference was very small. In a previous study, differences in aggressiveness were not found in a sample of four *F. meridionale* and two *F. graminearum* strains inoculated on stalks of seedlings (25-day-old plants) in the greenhouse (Kuhnem Júnior et al. 2013). The difference in our field study may be related to the influence of environmental factors or host age, both of which could increase susceptibility to a less aggressive pathogen. There may be other reasons for the dominance of *F. meridionale* on maize stalks, including inoculum availability and differential responses to environmental factors.

Resistance to GSR and GER in maize is complicated, as it is influenced both by genetic backgrounds and environmental factors (Mesterházy et al. 2012; Yang et al. 2010). Previous work has suggested that GSR has a direct relationship with GER, and that breeding for resistance should take both diseases into account (Mesterházy et al. 2012). Thus, GSR was shown to enhance

development and drying of the ear by interrupting the water supply, and this in turn reduced levels of ear rot by more than half (Mesterházy et al. 2012). Our study found no correlation in the degree of aggressiveness of individual isolates to ears versus stalks. Previous study has also found no correlation between GER and seedling blight (Kuhnem et al. 2015). It would be instructive to further explore pathogenicity by these two species by using a larger set of maize cultivars, and co-inoculation with both diseases at once to study potential interactions.

It is important to consider that aggressiveness-related traits alone may be insufficient to explain the dominance of one species over the others in the field, as other stages of the disease cycle (e.g. sexual reproduction, dispersal, etc.) may also play important roles in shaping species composition. Pathogen aggressiveness is a complex trait, not only because of its quantitative inheritance, governed by multiple genes with additive effects, but also because it is greatly influenced by interaction with environmental factors (Cumagun and Miedaner 2004). We assessed visual severity which was a consequence of the ability of the pathogen to colonize the ears after inoculation, and which can be influenced by environmental conditions. For example, previous studies using a sample of strains (n = 20) suggested higher sensitivity to temperature of *F. meridionale* compared with *F. graminearum* (Kuhnem et al. 2016). Thus, differences in prevailing temperature may be of influence, which should be investigated. *In vitro* studies showed that *F. graminearum* was able to produce perithecia and ascospores faster than other FGSC species *in vitro*, under optimum and constant temperatures (Liu et al. 2017; Nicolli et al. 2018). The higher degree of sensitivity to changes in temperature of *F. meridionale* versus *F. graminearum* may provide some advantage to the former during mixed infections in warmer environments (Kuhnem et al. 2016). It is possible that the amount of *F. meridionale* airborne inoculum is higher than *F. graminearum* during maize flowering, which can be confirmed by collecting airborne spores over the maize canopy. Although *F. graminearum* is dominant on wheat, a few FGSC species, including

F. meridionale, were collected in the air above the canopy during wheat flowering (Del Ponte et al. 2015).

The results of this study contribute new data and hypotheses on the biology of these species in subtropical and tropical conditions in Brazil and lay a foundation for further investigations of the mechanism of the predominance of *F. meridionale* associated with maize. Further studies using large sample of strains challenging both maize and wheat and looking into other phenotypic traits such as asexual and sexual reproduction under different environmental conditions will help to increase our understanding of ear and stalk rot epidemics.

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SUPPLEMENTARY MATERIAL

Table S1. Summary information for 41 arbitrarily selected isolates representing *Fusarium graminearum* (*n* = 16) and *F. meridionale* (*n* = 25) obtained from naturally infected maize kernels and symptomatic wheat heads in surveys of commercial fields in southern Brazil from 2009 to 2011.

Specie/ Genotype	Id.	Host	Year	Municipality	State
Fgra	188	wheat	2009	Panambi	RS
Fgra	189	wheat	2009	Ijuí	RS
Fgra	190	wheat	2010	Coxilha	RS
Fgra	191	wheat	2007	Cruz Alta	RS
Fgra	192	wheat	2007	Ernestina	RS
Fgra	193	wheat	2010	Tapejara	RS
Fgra	194	wheat	2011	Não-me-Toque	RS
Fgra	195	wheat	2011	Palmeira das Missões	RS
Fgra	196	wheat	2010	Caseiros	RS
Fgra	197	wheat	2010	Coxilha	RS
Fgra	07	wheat	2010	Estação	RS
Fgra	199	wheat	2011	Ijuí	RS
Fgra	15	maize	2011	Bom Jesus	RS
Fgra	205	maize	2011	Vacaria	RS
Fgra	207	maize	2011	Bom Jesus	RS
Fgra	209	maize	2011	Bom Jesus	RS
Fmer	01	wheat	2009	Ernestina	RS
Fmer	02	wheat	2009	Coxilha	RS
Fmer	03	wheat	2007	Nonoai	RS
Fmer	04	wheat	2011	Marau	RS
Fmer	05	wheat	2011	Sertão	RS
Fmer	198	wheat	2010	Água Santa	RS
Fmer	06	wheat	2010	Tapejara	RS

Fmer	08	wheat	2009	Santa Bárbara do Sul	RS
Fmer	21	wheat	2007	Nonoai	RS
Fmer	09	maize	2011	Marechal Cândido Rondon	PR
Fmer	10	maize	2011	Ponta Grossa	PR
Fmer	11	maize	2011	Irati	PR
Fmer	12	maize	2011	Casca	RS
Fmer	13	maize	2011	Eldorado do Sul	RS
Fmer	14	maize	2011	Bom Jesus	RS
Fmer	16	maize	2011	Taguaí	SP
Fmer	17	maize	2011	Paranapanema	SP
Fmer	18	maize	2011	Alambari	SP
Fmer	200	maize	2011	Ponta Grossa	PR
Fmer	201	maize	2011	Ponta Grossa	PR
Fmer	202	maize	2011	Ponta Grossa	PR
Fmer	203	maize	2011	Palmeira	PR
Fmer	204	maize	2011	Palmeira	PR
Fmer	206	maize	2011	Vacaria	RS
Fmer	208	maize	2011	Bom Jesus	RS

[a] Species and trichothecene genotype identified using the multilocus genotype method (Ward et al. 2008). Fgra = *F. graminearum* with 15-acetyl-deoxynivalenol genotype, Fmer = *F. meridionale* with nivalenol genotype.
[b] Isolates were obtained either from maize kernels (Kuhnem et al. 2016) or symptomatic wheat heads (Del Ponte et al. 2015) in previous surveys.
[c] Brazilian states: RS = Rio Grande do Sul, PR = Paraná, SP = São Paulo.

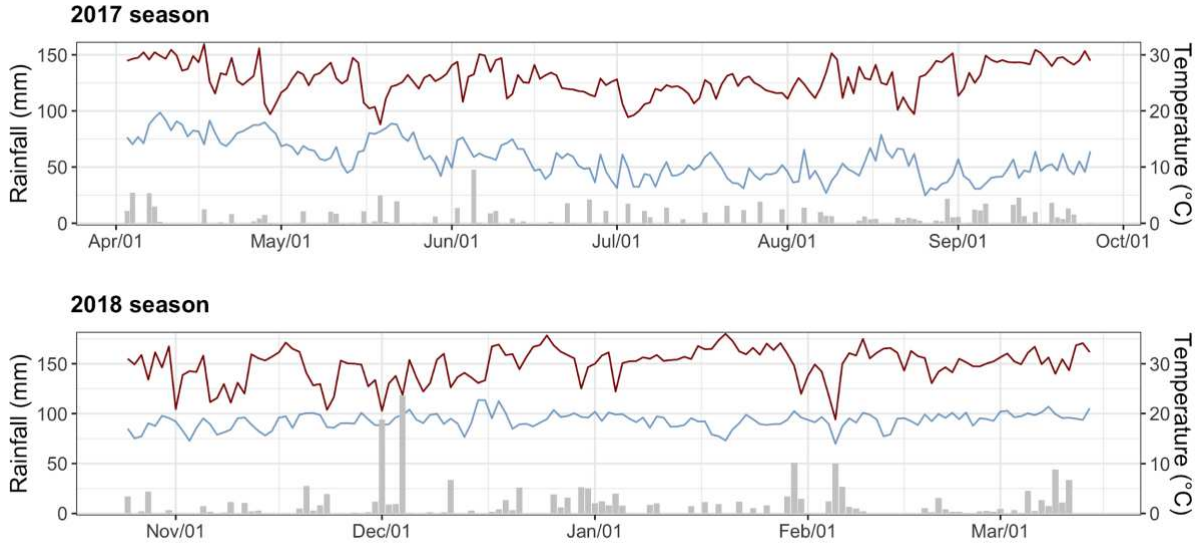

Figure S1. Daily values of meteorological data recorded by an automatic meteorological station located within the field trial. Minimum and maximum air temperature (°C) are represented in red and blue solid lines, respectively. Precipitation (rain plus irrigation) is represented by vertical bars. During the 2017 growing season, SupremoViptera® (Syngenta) and RB9004 PRO2® (KWS sementes) maize hybrids were planted on 4th and 25th of April, inoculated on 15th of June and 7th of August and harvested on 18th of August and 25th of September, respectively. The maize hybrids MG580PW® (Dow AgroSciences) and BM820® (Sementes Biomatrix) were planted planted on 25th of October and 20th of November 2017, inoculated on 5th of January and 9th of February and harvested on 11th of February and 15th of March during 2018 growing season, respectively.

ARTICLE 3

Unraveling shifts in dominance of *Fusarium meridionale* and *F. graminearum* on maize versus wheat in Brazil: a multivariate phenotypic analysis

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ABSTRACT

Machado, F. J., Vieira, A. V., Schmale III, D. G., Del Ponte, E. M., Vaillancourt, L. J. 20XX. Unraveling shifts in dominance of *Fusarium meridionale* and *F. graminearum* on maize versus wheat in Brazil: a multivariate phenotypic analysis. *XXXXX*: xx-xx.

Fusarium head blight (FHB) and Gibberella ear and stalk rot (GER and GSR) are diseases of worldwide importance affecting wheat and maize, respectively. In Brazil, a handful of *Fusarium graminearum* species complex (FGSC) members cause these diseases, but the dominant species varies according to the host. We undertook a comparison of pathogenic and saprophytic fitness-related traits for a large number of strains representative of the two species and hosts of origin in order to enhance understanding for host association. A collection of 45 strains, including 18 *F. graminearum* (12 from wheat and six from maize) and 27 *F. meridionale* (nine from wheat and 18 from maize), was compared in relation to 17 phenotypic traits. Although there was significant intraspecies variation for most traits, strains were strongly structured by species regardless of the host of origin, based on a multivariate analysis. *F. graminearum* was a more aggressive pathogen of wheat, and produced more abundant macroconidia, perithecia, and ascospores in culture. All *F. graminearum* strains produced primarily deoxynivalenol (DON), and more of its acetylated form 15ADON versus 3ADON, either in rice cultures or on wheat heads. In contrast, *F. meridionale* was a more aggressive colonizer of maize silks, and grew faster in culture. All *F. meridionale* strains produced mainly nivalenol (NIV) both *in vitro* and *in planta*, with the exception of two strains from maize that produced more DON than NIV in wheat heads. Overall, traits related to sexual or asexual reproduction and aggressiveness to wheat heads contributed the most to distinguish isolates of *F. graminearum*. On the other hand, aggressiveness on maize silks and mycelial growth was highly associated with *F. meridionale* strains. This study provides a baseline for improving our knowledge of the biology and ecology of FGSC species infecting wheat and maize.

Key-words: Fusarium head blight, Gibberella ear rot, Gibberella stalk rot.

INTRODUCTION

A handful of species of the *Fusarium graminearum* species complex (FGSC) are pathogens of major cereal crops. In wheat and maize, they cause flowering diseases known as Fusarium head blight (FHB) (a.k.a. wheat scab) and Gibberella ear rot (GER), respectively (Goswami and Kistler 2004; Kuhnem et al. 2015; Munkvold 2003a). These diseases, together with Gibberella stalk rot (GSR) of maize also caused by the same pathogens, cause significant economic losses due to grain yield reduction. In addition, the flowering diseases degrade crop value via contamination of grain with mycotoxins, including deoxynivalenol (DON) which is regulated for maximum tolerance limits (McMullen et al. 2012; Sutton 1982). *Fusarium graminearum* is the most common species causing FHB in wheat and barley worldwide. This species produces mainly DON, although there are some geographically-defined subpopulations that produce more nivalenol (NIV) (Del Ponte et al. 2015; Kelly and Ward 2018). *Fusarium meridionale* strains are typically NIV-producers, and are regionally important as causal agents of GER, and GSR in South America (Kuhnem et al. 2016; Sampietro et al. 2011) and Nepal (Desjardins and Proctor 2011). *Fusarium meridionale* is the second most common species causing FHB in wheat in Brazil, after *F. graminearum* (Del Ponte et al. 2015). Several other *Fusarium* species, both NIV-producing (*F. graminearum*, *F. asiaticum*, *F. cortaderiae,* and *F. austroamericanum*) and non-producing (*F. culmorum*, *F. cerealis* and *F. poae*), also cause disease in maize in other regions of the world (Basler 2016; Kuhnem et al. 2016; Lee et al. 2012; Ndoye et al. 2012). FGSC members produce zearalenone (ZON) in addition to the type-B trichothecenes, and all these compounds are extremely harmful for human and animal health (Chen et al. 2019). Maximum limits of DON on grains, food and feed are well established for several countries (van Egmond et al. 2007). Although NIV-producing species are also found

associated with maize and wheat, NIV has not been regulated by any county so far (Ferrigo et al. 2016; Park et al. 2018).

It is not known why one species within the FGSG complex tends to dominate among isolations from specific cereals in some regions, e.g. *F. meridionale* on maize in Brazil (Del Ponte et al. 2015; Kuhnem et al. 2016), or *F. asiaticum* on rice in both China and Brazil (Gomes et al. 2015; Zhang et al. 2012). One possibility could relate to differences in saprophytic fitness in particular environments. For example, *F. asiaticum* from rice was recovered in higher frequencies from rice straw than the other species (Lee et al. 2009). Although *F. meridionale* is largely dominant in corn stubble and stalks (Kuhnem et al. 2016), strains of this species are less fertile than *F. graminearum* (Nicolli et al. 2018), at least under artificial inoculation conditions, and this would not be expected to favor the build-up and dispersal of *F. meridionale* under conditions of mixed inoculum. However, that single study did not look at aspects of fitness beyond sexual development. A second possibility is increased aggressiveness of some species to specific hosts. A previous study with a relatively small sample of Brazilian strains of *F. graminearum* and *F. meridionale* from wheat ($n < 10$) showed that they were similarly aggressive towards this host (Spolti et al. 2012a; Kuhnem Júnior et al. 2013; Nicolli et al. 2015, 2018). In the first chapter of this dissertation, we demonstrated that a mixture of two *F. meridionale* isolates were more aggressive (higher disease severity) towards maize ears than a mixture of two *F. graminearum* strains. In the second chapter, we increased the sample size ($n \geq 18$ strains) of both species and included isolates from both crops. We also evaluated the isolates separately rather than in mixtures. The same pattern was confirmed, showing that *F. meridionale* was, on average, twice as aggressive on ears compared with *F. graminearum*. This finding could explain its prevalence among strains recovered from maize kernels (67%) compared to *F. graminearum* isolates (18%) (Kuhnem et al. 2016). However, *F. graminearum* was 14% more aggressive to maize stalks than *F. meridionale,* so aggressiveness cannot explain the relative over-representation of *F. meridionale* (53%) among

strains recovered from corn stubble (Kuhnem et al. 2016). We hypothesize that the dominance by *F. meridionale* on maize stubble might be due to differential saprophytic abilities, or differences in the prevalence on wheat in the maize-wheat rotation cycle. None of these previous studies have included inoculations of both hosts with the same strains, thus conclusions on relative host preference could not be made. Our goal was to more fully address these questions by conducting a multi-phenotypic study of a representative sample of strains from both hosts, including inoculations of both hosts with the same strains, together with a more complete evaluation of other traits that have been related to the saprophytic stage of the disease cycle (Del Ponte et al. 2015; Gomes et al. 2015; Kuhnem et al. 2016; Yang et al. 2018; Zhang et al. 2016). In this chapter, we used the same isolates from previous chapters, plus a few additional representatives, to inoculate wheat plants and measured aggressiveness and toxin production, and also compared their growth, fungicide resistance, toxin production, and the production of sexual and asexual spores *in vitro*.

MATERIALS AND METHODS

Forty-five isolates were selected from a larger collection obtained from maize kernels (Kuhnem et al. 2016) and symptomatic wheat heads (Del Ponte et al. 2015) during surveys of commercial fields in southern Brazil from 2009 to 2011 (Fig. 1). Eighteen isolates of *F. graminearum* (12 from wheat and six from maize) and 27 isolates of *F. meridionale* (nine from wheat and 18 from maize) were selected to represent the geographic and temporal diversity of the collection (Table S1). The isolates were previously assigned to species and trichothecene genotype via a MLGT assay using a Luminex flux cytometer (Ward et al. 2008) and had been preserved as a permanent collection for future studies. After recovery from storage, the identities of the isolates were confirmed using the Fg16F/R primer set (Nicholson et al. 1998). These primer set provide different fragment sizes that identify isolates as either *F. meridionale* (~500 bp) or *F. graminearum* (~450 bp) (Astolfi et al. 2012; Castañares et al. 2016; Del Ponte et al. 2015; Nicholson et al. 1998).

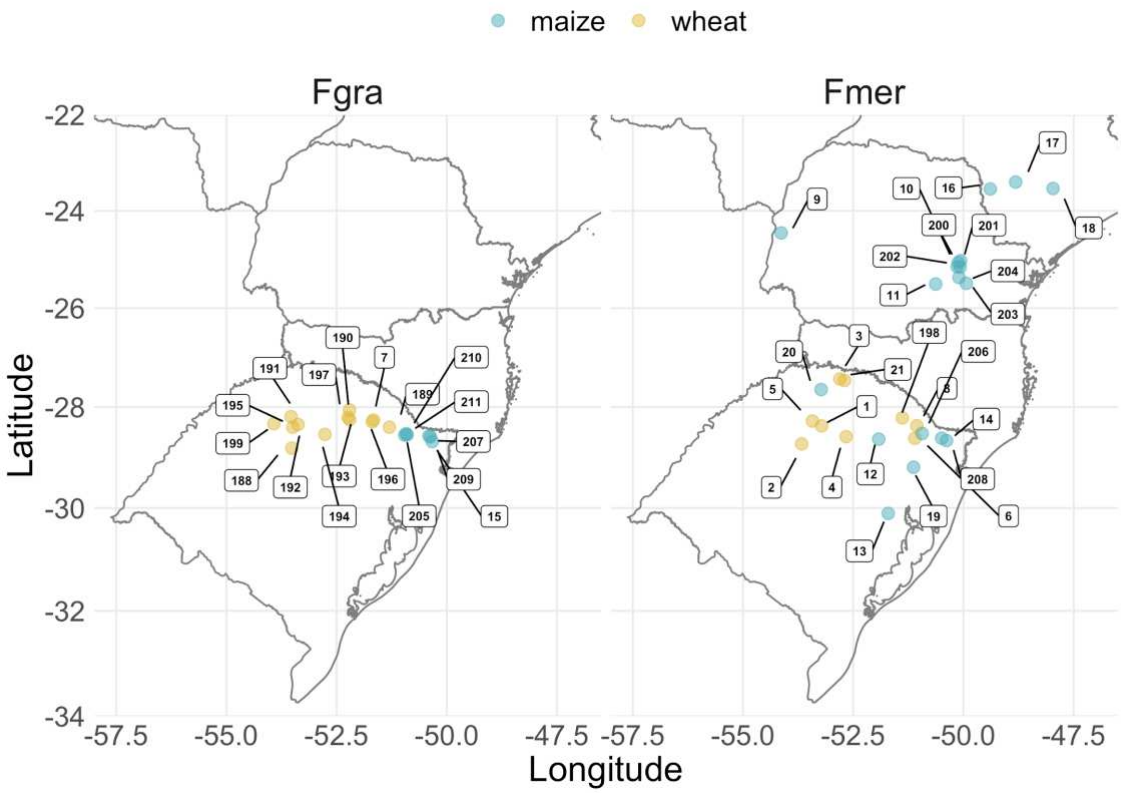

Figure 1. Geographic origin of 18 *Fusarium graminearum* isolates (n_{maize} = 6; n_{wheat} = 12) and 27 *F. meridionale* isolates (n_{maize} = 18; n_{wheat} = 9) obtained from symptomatic wheat heads and maize kernels in Southern Brazil from 2009 to 2011. Number associated with each datapoint represents the isolate identification in our collection.

Saprophytic traits

Mycelial growth. Three equidistantly positioned colonies were formed by placing 5-µl drops of a spore suspension (10,000 macroconidia/ml) onto a 9-cm PDA plate as described previously (Zhan and McDonald 2011) with some modifications (Spolti, Del Ponte, Cummings, et al. 2014a). The media were prepared as a single batch and poured into 9-cm plastic Petri dishes (15 ml). Inoculated plates were allowed to dry before being randomly placed in the growth chamber. The cultures were incubated at 23 °C in darkness for 5 days. Radial mycelial growth was obtained by averaging two perpendicular measurements. The entire assay was repeated three times.

Asexual spore production. Mycelial plugs from a 5-day-old PDA plate were used to inoculate Mung Bean Agar plates (MBA: 40 g of mung bean/liter, placed in boiling distilled water for 23 min [~50% of seed pericarps split while cooking] filtered through two layers of cheesecloth, adjusted to 1 liter, 15 g of agar) (Evans et al. 2000). Each isolate was cultured for seven days at 23 °C under constant lights. Three agar discs (6-mm in diameter) were taken from the edges of each colony and added to a microcentrifuge tube containing one milliliter (ml) of sterile water. Spores were harvested by vortexing each tube for 20 s (Nicolli et al. 2018). The macroconidial concentration was quantified by using a hemocytometer and expressed as the number of macroconidia per ml. Each plate was considered as one replicate (three plates per isolate) and the entire assay was repeated once.

Sexual fertility. The sexual fertility was assessed based on the ability of each isolate to produce perithecia and ascospores on carrot agar following a standard protoco1 with some adaptations (Cavinder et al. 2012). Carrot agar plates (6.0-cm-diameter) were inoculated by placing a 5-µl drop of a spore suspension (1×10^4 macroconidia/ml) of each isolate in the center. Plates were incubated in a growth room at 23°C under constant luminosity until the mycelium reached the edge of the plate (~ 4 days). Aerial mycelia were gently removed using a toothpick and then 1 ml of 2.5% Tween 80 in water was distributed with a sterile plastic micro-pestle. Plates were returned to the light. At 21 days after induction, perithecia were quantified under a stereomicroscope within a 1-cm^2 area at two different positions on the plate and expressed as mean perithecia per cm^2. Ascospore production was measured 21 days after induction by applying 5 ml of sterile deionized water and gently rubbing the plate surface with a sterile plastic micro-pestle. Ascospore suspensions were filtered through two layers of cheesecloth. Ascospore concentration was estimated by using a hemocytometer, and expressed as the number of ascospores per ml. Each

plate was considered as one replicate and placed on different benches in the growth room (blocks, three plates per isolate). The entire assay was performed once.

Pathogenic and toxigenic traits

Maize silk infection assay. Maize silk infection was assessed using a standard protocol with some modifications (Seong et al. 2005). Susceptible sweet corn hybrid Golden Jubilee was sown in the greenhouse to produce fresh ears every week. Briefly, three seeds were planted in 25-cm pot filled with a mixture of three parts ProMix BX (Premiere Horticulture Ltd., Riviere du Loup, PQ, Canada) and one-part topsoil. Maize plants were grown in greenhouse with a 14 h photoperiod and temperatures ranging from 25 to 28 °C. Seedlings were thinned to two plants per pot and fertilized weekly with a solution of Peters 20-20-20 fertilizer (Scotts-Sierra Horticultural Product Co. Marysville, OH). At the silking stage, primary maize ears were harvested, and the exposed portions of silks were trimmed off. In the laboratory, 4-5 maize silks were sliced into 5-cm sections and the sections were bundled and aligned on a water-soaked Whatman No 1 filter paper. Three silk bundles were positioned alongside each other inside a 90-mm petri dish. A mycelial agar plug taken from the edge of a 7-day-old culture of each isolate growing on MBA (6-mm in diameter) was placed upside down covering the lower ends of the silks. Uninoculated MBA plugs were used as negative controls. Plates were placed inside a closed plastic box to maintain high humidity. Boxes were incubated in a growth room at 23 °C under constant light for 4 days. Infection was assessed by measuring the extent of tissue discoloration using a digital caliper. The entire assay was repeated twice.

Aggressiveness on wheat heads. Seeds of spring wheat variety Wheaton, which is highly susceptible to FHB, were sown in cone containers filled with a mixture of ProMix BX and topsoil (3:1). Wheat plants were grown in greenhouse with a 14 h photoperiod and temperatures ranging

from 25 to 28 °C. Seedlings were fertilized weekly with a solution of Peters 20-20-20 fertilizer (Scotts-Sierra Horticultural Product Co. Marysville, OH) and kept in the greenhouse until flowering. A spore suspension was prepared by growing each isolate on mung bean agar (MBA) for 7-14 days under constant lights. The macroconidia suspensions were filtered through two layers of cheesecloth. The concentration of each macroconidial suspension was then quantified using a hemocytometer adjusted to $1 \times 10_4$ macroconidia/ml. The wheat heads were inoculated with a 10-µl drop of the spore suspension ($1 \times 10_4$ macroconidia/ml) placed inside each lateral floweret of the central spikelet at early- to mid-anthesis. Each head was individually covered with a plastic bag for 24 h and kept in a contained growth chamber set for 25 °C and a 14 h photoperiod until harvest (plant maturity). FHB severity was assessed at four, seven- and 10-days post-inoculation, as the number of FHB symptomatic spikelets per inoculated spike. Three replicates (individual head) were used per isolate. The experiment was performed three times.

Trichothecene production in planta. Harvested wheat heads from the aggressiveness assay were dried at room temperature and kept in a cold room (4 °C) until analysis. Mycotoxin production by each of the 45 isolates was determined by bulking the samples from each experiment and considered as a replicate. First, the entire wheat heads were ground to obtain at least a 5-g sample of each replicate. The ground samples were sent to the Virginia Tech Deoxynivalenol (DON) testing Lab. The amount of DON and its acetylated forms (15ADON and 3ADON), NIV and ZON were quantified using a gas chromatography–mass spectrometry method as described previously (Fuentes et al. 2005; Mirocha et al. 1998).

Trichothecene production in rice. Mycotoxin production in rice culture was determined using a standard protocol with some modifications (Spolti et al. 2014b; Puri and Zhong 2010; Burlakoti et al. 2008; Walker et al. 2001). Rice grains (30g) were soaked in 13 ml of sterile deionized water

overnight (~10h) in a 250-ml Erlenmeyer flask. All flasks were autoclaved for 30 minutes and inoculated with a 500 µL of a spore suspension (1×10^4 macroconidia/ml) of each isolate. Three flasks (replicates) were prepared for each isolate and all isolates were grown at the same time in the same location. The flasks were shaken manually every two day to allow the fungus to evenly colonize the substrate. The cultures were incubated for 28 days at 23 °C in the dark. The entire assay was performed once. The colonized rice cultures were transferred into a 50-ml centrifuge tube, frozen overnight at -80 °C, and then lyophilized for 72 h at -40 °C. The rice cultures were ground using a coffee grinder and a 5-g sample was sent to Virginia Tech Deoxynivalenol (DON) testing Lab, for mycotoxin analysis. The amounts of DON and its acetylated forms (15ADON and 3ADON), NIV, and ZON were quantified using a gas chromatography–mass spectrometry method as described previously (Fuentes et al. 2005; Mirocha et al. 1998).

Tebuconazole sensitivity

Sensitivity to tebuconazole (Folicur 3.6F; 38.7% active ingredient; Bayer Cropscience, Research Triangle Park, NC), was estimated by measuring radial growth of the isolates on PDA adjusted to different fungicide concentrations in three replicates each as described previously (Spolti et al. 2012b; Spolti et al. 2014b; Chen et al. 2007). The tested concentrations were: 0 (non-amended agar - PDA), 0.5, 1.0, 2.0, 4.0 and 8.0 µg a.i./ml. Plates were incubated at 23°C in darkness for 5 days. Colony diameters were measured in two perpendicular directions using a digital caliper and the original plug diameter were subtracted. Effective concentration leading to a 50% reduction of mycelial growth (EC_{50}) were calculated based on the linear regression analysis between the relative mycelial growth inhibition (percent) and the log-transformed fungicide concentrations. The entire experiment was repeated twice.

Data analysis

All experiments were conducted as a completely randomized design with three replicates. Data from assays conducted two or three times were combined for analysis. The overall mean and the standard deviation and Cohen's *d* were estimated using 'effsize' R package (Torchiano 2019). A multivariate analysis of variance (MANOVA) was performed. Mycotoxin data were not included in the multivariate analysis because not all isolates produced the same mycotoxin. Prior to the analysis, all variables were transformed [log(X + 1)] for normality and homoscedasticity. Principal components analysis (PCA) was performed using the mean values of the variables for each isolate, averaged over replicates and experiment. The contribution of each variable to each principal component was also estimated. A correlogram was made using the overall means for each pair of variables using the package 'corrplot' (Wei and Simko 2017). The packages 'FactoMineR' (Lê et al. 2008) and 'factoextra' (Kassambara and Mundt 2017) were used to PCA. All analyses were run in R (R Core Team 2019).

RESULTS

Saprophytic traits

The means of mycelial growth did not differ according to the host of origin for *F. graminearum* (maize = 27.18 ± 1.94 mm and wheat = 27.65 ± 2.61 mm) or *F. meridionale* strains (maize = 31.17 ± 3.52 mm and wheat = 30.44 ± 1.93 mm) (Fig. 2A). In general, *F. meridionale* strains grew faster than *F. graminearum* strains (Table 1). In contrast, *F. graminearum* produced more macroconidia than *F. meridionale* isolates (Table 1). Maize isolates produced more spores than isolates from wheat for both *F. graminearum* (102.94 ± 48.34 × 10_4 macroconidia/ml) and *F. meridionale* (20.67 ± 32.49 × 10_4 macroconidia/ml) (Fig. 2B).

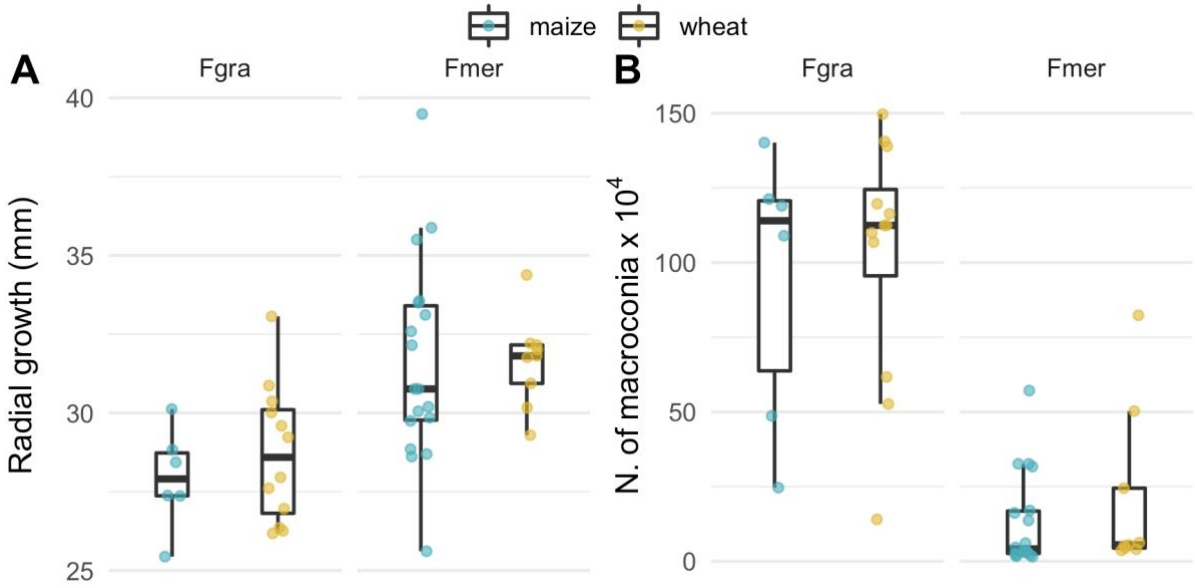

Fig. 2. (A) Average radial growth on PDA, and **(B)** macroconidial production on MBA at 23°C of a sample of 45 isolates obtained from wheat heads or maize kernels. In total, 18 *F. graminearum* isolates (n_{maize} = 6; n_{wheat} = 12) and 27 *F. meridionale* isolates (n_{maize} = 18; n_{wheat} = 9) were utilized in this work. Datapoints for each isolate, averaged over three replicates of two independent experiments combined.

Table 1. Summary information of saprophytic traits of 45 *Fusarium graminearum* and *F. meridionale* isolates obtained from either maize kernels or symptomatic wheat heads in Southern Brazil from 2009 to 2011.

	Species[a]				
Trait	*n*	F. gra.	*n*	F. mer.	Cohen's *d*[b]
Mycelial growth[c]	108	27.49 ± 2.41	162	30.93 ± 3.10	-1.21
Spore production[d]	108	99.89 ± 49.41	162	15.58 ± 28.32	2.21
Perithecial production[e]	54	305.31 ± 185.72	81	119.61 ± 103.00	1.31
Ascospore production[f]	54	296.20 ± 343.01	81	16.85 ± 27.49	1.28
EC_{50} [g]	36	0.61 ± 0.45	54	0.21 ± 0.15	1.30

[a] Isolates were simultaneously assigned to species and trichothecene genotype using MGLT assay (Ward et al. 2008). In total, 18 *F. graminearum* isolates ($n = 6$ from maize, $n = 12$ from maize) and 27 *F. meridionale* isolates ($n = 18$ from maize, $n = 9$ from maize) isolates were used in this study. Data are shown as means ± standard deviation.

[b] Cohen's *d.* Small ($d > 0.2$), medium ($d > 0.5$), and large ($d > 0.8$) effect sizes (Gent et al. 2018).

[c] Mycelial growth (mm) were determined on potato dextrose agar (PDA) at 23 °C incubated for five days under constant lights.

[d] Macroconidial production ($\times 10^4$ macroconidia/ml) on Mung Bean Agar (MBA) at 23 °C after seven days of incubation.

[e] Perithecial production (perithecia/cm^2) on carrot agar (CA) incubated at 23 °C for 21 days under constant lights.

[f] Ascospores (x10^4 ascospores/ml) harvested and counted from CA media at the 21st day.

[g] Effective concentration of tebuconazole (µg/ml) that reduces 50% of the mycelial growth of each of the isolates.

All but one *F. meridionale* strain was able to produce perithecia *in vitro*. There was substantial intraspecies variation in both perithecial and ascospore production (Fig. 3A-B). Two *F. meridionale* isolates produced protoperithecia on carrot agar, but did not produce ascospores (Fig. 3B). Overall, *F. graminearum* isolates produced 2.5 times more perithecia and 17.5 times more ascospores than *F. meridionale* isolates (Table 1). *F. graminearum* from maize and wheat produced 271.28 ± 182.65 perithecia/cm^2 ($n = 18$) and 322.32 ± 187.44 perithecia/cm^2 ($n = 36$), respectively. *F. graminearum* from wheat were much more fertile than those from maize, 398.61 ± 378.28 × 10^4 ascospores/ml ($n = 36$) and 91.39 ± 74.61 × 10^4 ascospores/ml ($n = 18$), respectively.

F. meridionale strains from maize and wheat produced 111.99 ± 91.49 perithecia/cm2 (n = 54) and 134.85 ± 123.36 perithecia/cm2 (n = 27) respectively (Fig. 3A). Thus, *F. meridionale* strains regardless of the host of origin produced fewer perithecia than *F. graminearum* strains, but they produced a similar number of ascospores, 19.81 ± 32.42 (n = 54) and 10.93 ± 11.35 × 104 ascospores/ml (n = 27), respectively (Fig. 3B).

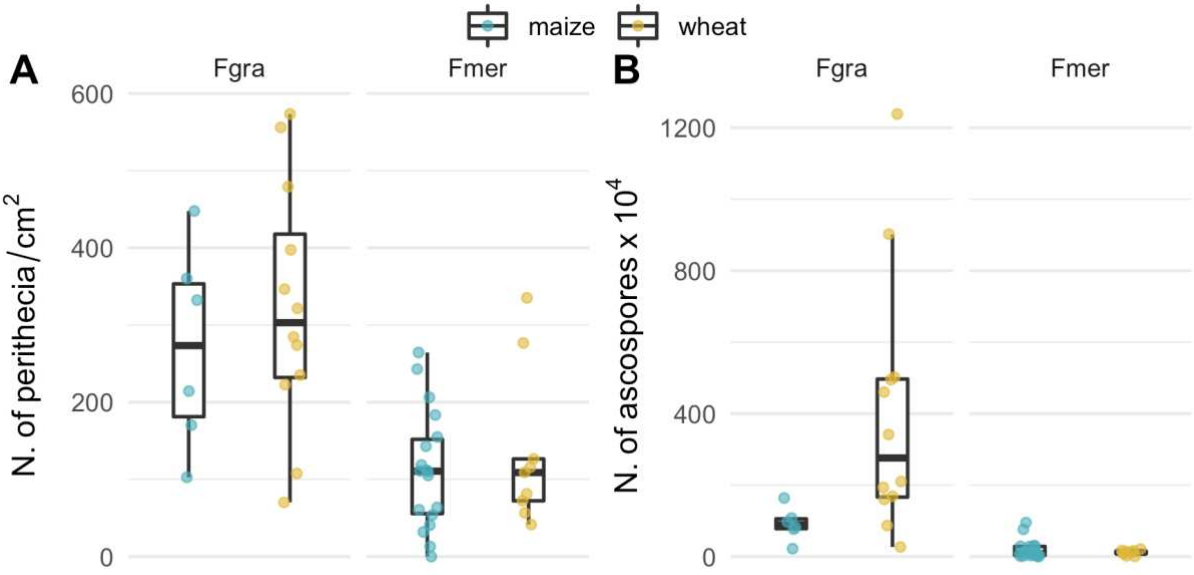

Fig. 3. (A) Perithecial and **(B)** ascospore production on carrot agar incubated for among a sample of 45 isolates obtained from wheat heads or maize kernels. In total, 18 *F. graminearum* isolates (n_{maize} = 6; n_{wheat} = 12) and 27 *F. meridionale* isolates (n_{maize} = 18; n_{wheat} = 9) were utilized in this work. Datapoints for each isolate, averaged over three replicates of a single experiment.

Pathogenic traits

Aggressiveness on maize silks. Mean lesion lengths were 21.97 ± 9.56 mm (n = 36) and 26.87 ± 9.72 mm (n = 72) for *F. graminearum* isolates from maize and wheat, respectively. Mean lesion lengths for *F. meridionale* isolates from maize and wheat were 18.81 ± 11.38 mm (n = 108) and 19.29 ± 11.69 (n = 54) respectively (Fig. 4B). On average, the effect size when comparing *F. meridionale* to *F. graminearum* was considered medium (Cohen's d = 0.58). The former was 33% more aggressive than the latter (Table 2).

Fig. 4. Distribution of **(A)** Fusarium head blight severity (%) on susceptible spring wheat variety 'Wheaton' plants at 10 days post inoculation, and **(B)** lesion length on sweet corn hybrid Golden Jubilee silks among 18 *F. graminearum* isolates (n_{maize} = 6; n_{wheat} = 12) and 27 *F. meridionale* isolates (n_{maize} = 18; n_{wheat} = 9). Datapoints for each isolate, averaged over three replicates of three and two independent experiments combined, respectively.

Aggressiveness on susceptible wheat heads. All strains were able to produce typical FHB symptoms, but the disease severity at 10 days post inoculation varied greatly among strains (Fig. 4A), ranging from 9.16 to 40.99% and from 5.26 to 10.66% for *F. graminearum* and *F. meridionale*, respectively (Fig. 4A). The mean severities for *F. graminearum* isolates from maize and wheat were 20.07 ± 18.76% (n = 48) and 26.63 ± 21.20% (n = 94) respectively. Within *F. meridionale*, the mean severities for maize and wheat strains were 7.20 ± 3.17% (n = 142) and 6.67% 1.81 (n = 70), respectively (Fig. 4A). On average, *F. graminearum* isolates were 3+ times more aggressive on wheat heads than *F. meridionale* isolates (Table 2).

Toxigenic traits

Mycotoxin production in wheat heads. All *F. graminearum* strain produced primarily DON and small amounts of the two acetylated forms (Table 2). DON ranged from 53.64 to 209.68 µg/g (Fig.

5). The chemotype confirmed the previous PCR-genotype analysis: 15ADON was 6.5 times higher than 3ADON (Table 2). There were seven 15ADON strains from maize that produced trace levels of NIV (0.31 µg/g) (Fig. 5). Consistent with the PCR-genotyping, all *F. meridionale* isolates produced mainly NIV at levels 17-fold higher than *F. graminearum* isolates. Four *F. meridionale* strains were also producers of 15ADON and DON (Fig. 5). Exceptionally, two *F. meridionale* strains from maize produced even more DON than NIV (Fig. 5). Neither *F. graminearum* nor *F. meridionale* isolates produced detectable levels of ZON in wheat kernels (Table 2).

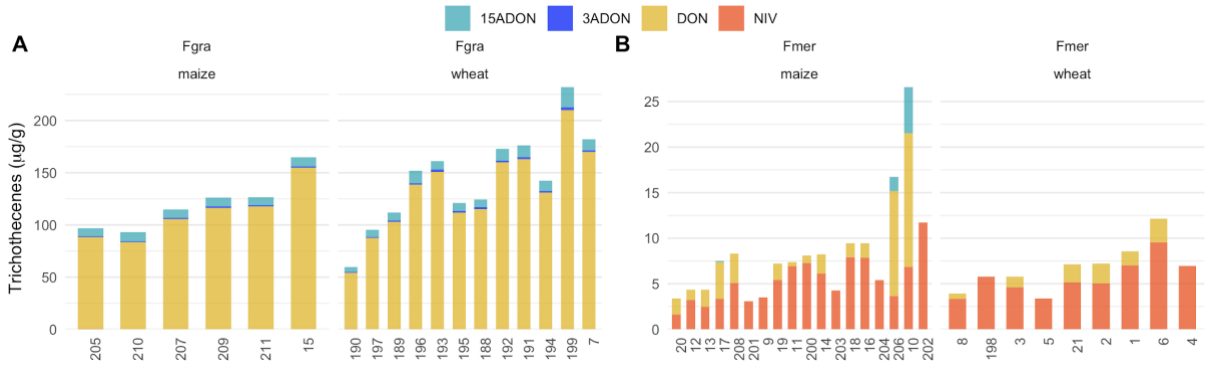

Fig. 5. Mean production of trichothecenes: deoxynivalenol (DON), 15-acetyl-deoxynivalenol (15ADON) and 3ADON and nivalenol (NIV) by (**A**) the 18 *Fusarium graminearum* and (**A**) 27 *F. meridionale* isolates inoculated into wheat heads (cv. Wheaton).

Table 2. Summary information of pathogenic and toxigenic traits of 45 *Fusarium graminearum* and *F. meridionale* isolates obtained from either maize kernels or symptomatic wheat heads in Southern Brazil from 2009 to 2011.

	Species[a]				
Trait	*n*	F. gra.	*n*	F. mer.	Cohen's *d*[b]
FHB severity (%)[c]	142	24.51 ± 20.58	212	7.02 ± 2.80	1.32
Silk infection (mm)[d]	108	25.24 ± 9.90	162	18.97 ± 11.45	0.58
Mycotoxins *in planta*[e]					
DON (μg/g)	54	125.51 ± 63.22	81	3.49 ± 6.60	2.34
15ADON (μg/g)	54	9.28 ± 4.28	81	1.34 ± 2.17	1.90
3ADON (μg/g)	54	1.42 ± 0.87	81	-	--
NIV (μg/g)	54	0.31 ± 0.21	81	5.34 ± 3.37	-1.55
ZON (μg/g)	54	-	81	-	-
Mycotoxins *in vitro*[f]					
DON (μg/g)	57	339.17 ± 340.96	78	-	--
15ADON (μg/g)	57	32.27 ± 27.31	78	-	--
3ADON (μg/g)	57	3.68 ± 3.79	78	3.02 ± 3.41	-0.18
NIV (μg/g)	57	7.49 ± 17.49	78	19.72 ± 22.41	-0.57
ZON (μg/g)	57	233.16 ± 527.75	78	26.99 ± 59.49	0.46

[a] Isolates were simultaneously assigned to species and trichothecene genotype using MGLT assay (Ward et al. 2008). In total, 18 *F. graminearum* isolates ($n = 6$ from maize, $n = 12$ from wheat) and 27 *F. meridionale* isolates ($n = 18$ from maize, $n = 9$ from wheat) isolates were used in this study. Data are shown as means ± standard deviation.

[b] Cohen's *d.* Small ($d > 0.2$), medium ($d > 0.5$), and large ($d > 0.8$) effect sizes (Gent et al. 2018).

[c] FHB severity (%) on 'Wheaton' plants inoculated by the single-floret method. Percentage of diseased spikelets in inoculated spikes at 10 days post inoculation.

[d] Lesion length (mm) on sweet corn hybrid Golden Jubilee silks.

[e] Trichothecene amount in grains from entire wheat heads (cv. Wheaton), inoculated in the greenhouse and averaged over three heads for each independent experiment; ppm = part per million (μg/g of samples). DON = deoxynivalenol; 3ADON = 3-acetyl-DON; 15ADON = 15-acetyl-DON; NIV = nivalenol.

[f] Mycotoxin amount (DON, 15ADON, 3ADON, NIV, and ZON = zearalenone) in rice cultures incubated at 23 °C for 28 days.

Mycotoxin production in rice cultures. All *F. graminearum* isolates were able to produce DON and 15ADON. Most (15 out of 18) isolates also produced 3ADON (Fig. 6), and 11 produced NIV.

Three of this last group of 11 were obtained from maize. All isolates produced ZON in rice cultures (Table 2, Fig. 6). All *F. meridionale* isolates, regardless of their host of origin, produced NIV (Fig. 6). In addition, one *F. meridionale* isolate from wheat produced DON, one from each host produced 15ADON, and seven from maize and five from wheat produced 3ADON (Fig. 6). ZON was produced by eight isolates from maize and three from wheat (Table 2, Fig. 6). One *F. meridionale* isolate from wheat produced all four mycotoxins on rice (strain 198, Fig. 6).

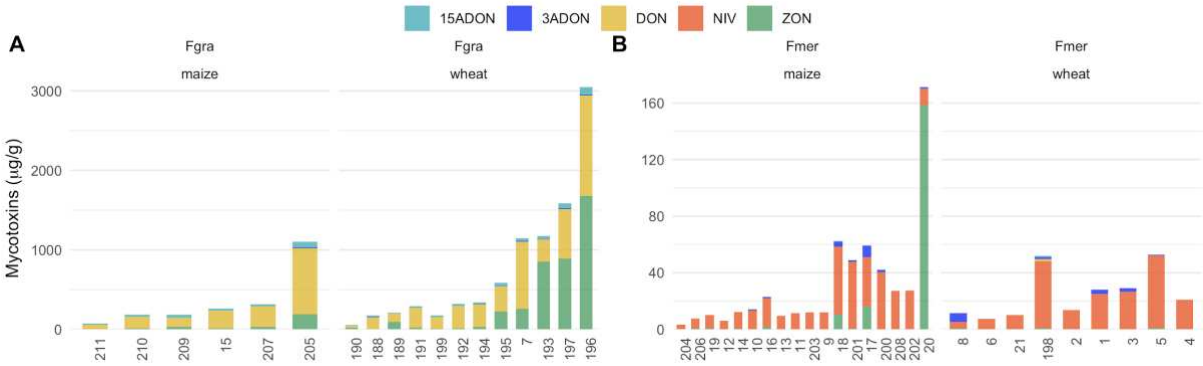

Fig. 6. Mean production of deoxynivalenol (DON), 15-acetyl-deoxynivalenol (15ADON) and 3ADON, nivalenol (NIV) and zearalenone (ZON) by each of the (**A**) 18 *Fusarium graminearum* and (**B**) 27 *F. meridionale* isolates in rice cultures.

Tebuconazole sensitivity

The mean EC_{50} value ± S.D. (standard deviation) for *F. graminearum* isolates from maize and wheat were 0.84 ± 0.55 µg/ml (n = 12) and 0.50 ± 0.36 µg/ml (n = 24) respectively. Only three *F. graminearum* isolates, all from maize, showed EC_{50} higher than 1.0 µg/ml (1.17, 1.19 and 1.46 µg/ml) (Fig. 7). For *F. meridionale*, mean EC_{50} were 0.21 ± 0.16 µg/ml (n = 36) and 0.20 ± 0.13 µg/ml (n = 18) for maize and wheat isolates, respectively (Fig. 7). All *F. meridionale* isolates showed EC_{50} lower than 0.5 µg/ml, with the exception of two maize strains (0.51 and 0.52 µg/ml) (Fig. 7). Overall, *F. graminearum* isolates were three times less sensitive to tebuconazole than *F. meridionale* isolates (Table 1).

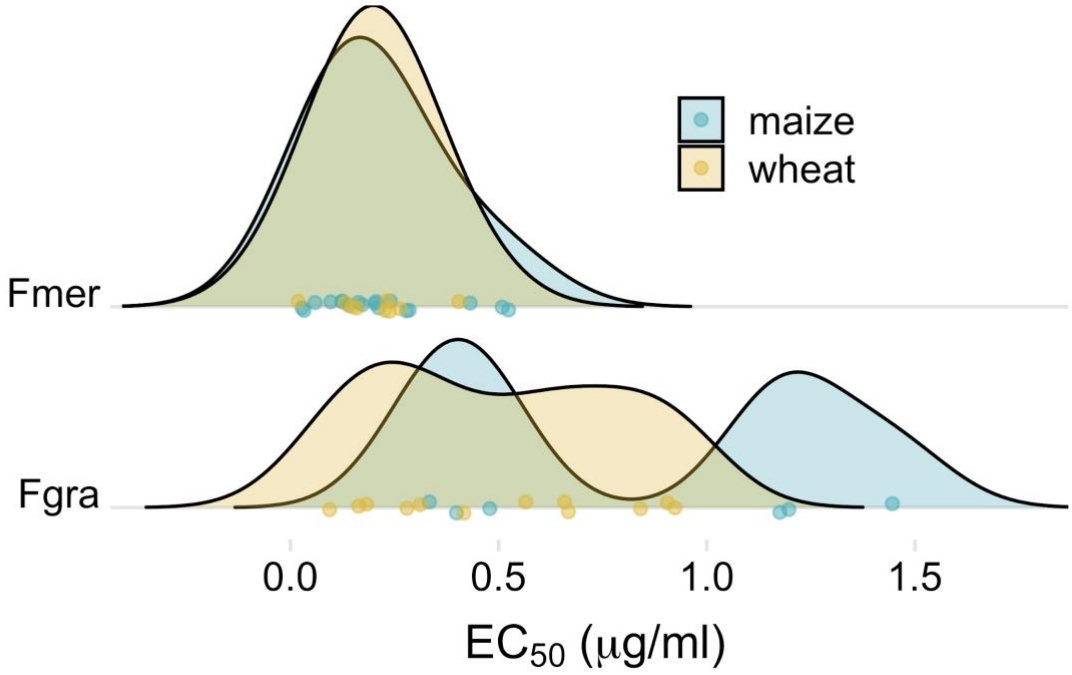

Fig. 7. Density plots of the effective concentration of tebuconazole that reduces 50% of the mycelial growth (EC_{50}) of a sample of 18 *F. graminearum* isolates (n_{maize} = 6; n_{wheat} = 12) and 27 *F. meridionale* isolates (n_{maize} = 18; n_{wheat} = 9). Datapoints for each isolate, averaged over the two independent experiments combined.

Overall species related fitness

Based on MANOVA results, there was weak evidence against the null hypothesis of interaction between species and host of origin (P = 0.078), contrasting with a strong effect of species (P < 0.001). The correlation analysis showed 75 significant associations (P < 0.05) over each of the 117 pairwise comparisons for all the 17 variables (Fig. S1). Overall, FHB severity at 10 dpi was highly associated with DON production in wheat heads (Fig. S1). NIV production was negatively associated with DON production either *in planta* or *in vitro* (Fig. S1). Mycelial growth was negatively associated with all other variables with the exception of NIV production, which also was negatively associated with all the other traits.

The PC analysis suggested that macroconidial production, aggressiveness to wheat heads at 10 dpi, and ascospore production contributed the most for the PC1: 24%, 23% and 16%

respectively of the total, with 49.4% of all variation explained by this first PC. The second PC explained 17.2% of the variation, with aggressiveness on maize silks and mycelial growth contributing 51% and 40%, respectively. The third PC explained 13.6% of the variation among isolates, with a contribution of 44% from the variable tebuconazole sensitivity (EC_{50}) and 34% from perithecial production. Those last two parameters were also negatively associated with PC2 (ρ = -0.20 and ρ = -0.13, respectively). Interestingly, mycelial growth was the only variable negatively associated with the first PC (ρ = -0.57). Cluster analysis showed that four isolates, including one *F. graminearum* from wheat, two *F. graminearum* from maize, and one *F. meridionale* from maize, did not group together with their respective clusters (Fig. S2). In general though, strains were structured by species (Fig S2).

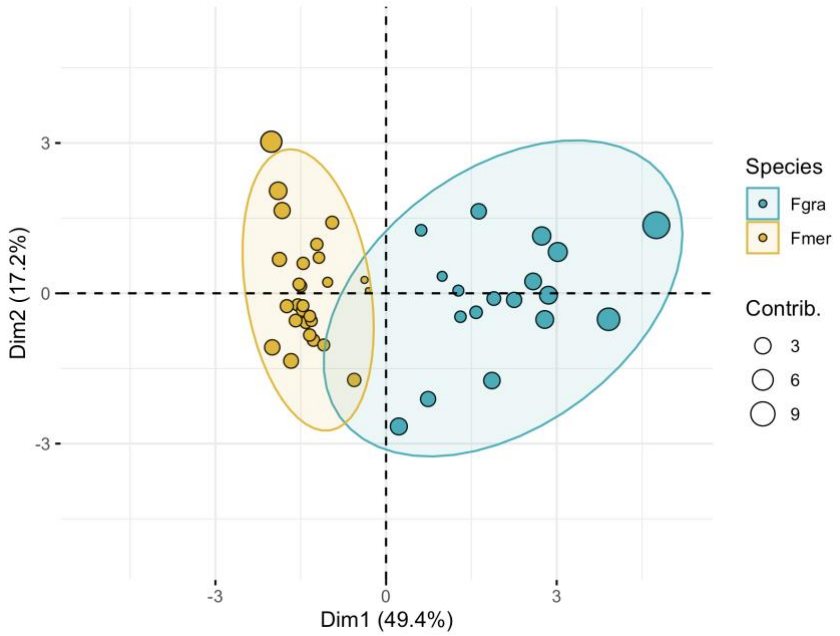

Figure 8. Scatterplot from the Principal Components Analysis of a sample of 18 *F. graminearum* isolates and 27 *F. meridionale* isolates (n_{maize} = 18; n_{wheat} = 9) obtained from naturally infected maize kernels and symptomatic wheat heads in surveys of commercial fields in southern Brazil from 2009 to 2011.

DISCUSSION

In this study we showed for the first time not only that *F. graminearum* and *F. meridionale* behave differently, but also that their host of origin plays an important role for most of saprophytic and pathogenic traits investigated. Results support the hypothesis that *F. graminearum* is the most prevalent FHB causal agent in Brazil because it is more aggressive on wheat than *F. meridionale* (Astolfi et al. 2012; Del Ponte et al. 2015; Scoz et al. 2009; Nicolli et al. 2018). We found that *F. graminearum* isolates were twice as fertile, and produced 17 times more ascospores and five times more macroconidia than *F. meridionale* isolates (Liu et al. 2017; Nicolli et al. 2018). The prior studies discussed the importance of these fitness parameters in the ecology and also the epidemiology of *F. graminearum* (Leplat et al. 2013; McMullen et al. 2012; Pereyra et al. 2004). Interestingly, while all the *F. graminearum* strains produced fertile perithecia on carrot agar, one *F. meridionale* isolate was unable to produce any perithecia, while two other produced only infertile protoperithecia. It has been previously reported that *F. meridionale*, a.k.a. *F. graminearum* lineage 2, has lower levels of female fertility compared to *F. graminearum* (Bowden and Leslie 1999).

Our study has also provided additional clues regarding the dominance of *F. meridionale* on maize ears (Kuhnem et al. 2016). We showed that *F. meridionale* isolates colonized maize silks more rapidly and more extensively than isolates of *F. graminearum*, regardless of their host of origin. In the first two chapters of this dissertation, we demonstrated that *F. meridionale* isolates were twice as aggressive to maize ears as *F. graminearum* isolates, and this could be related to their relative aggressiveness on silks. In this chapter we also showed that *F. meridionale* grew faster on PDA. Interestingly, a significant correlation has been reported between mycelial growth on PDA and the production of ascospores on corn stalks (Spolti et al. 2014a), and this could be related to the prevalence of this species in maize stubble.

We report for the first time the tebuconazole sensitivity for isolates of both species obtained from maize. Although no substantial differences were detected among isolates from different hosts, our results showed that *F. graminearum* isolates were less sensitive to tebuconazole than *F. meridionale* isolates, which is in agreement with previous studies with isolates from wheat (Machado et al. *unpublished*, Spolti et al. 2012b; Nicolli et al. 2018; Machado et al. 2017). Our findings are consistent with a previous study under greenhouse conditions which demonstrated a higher fungistatic effect of tebuconazole against *F. meridionale* in wheat heads compared to *F. graminearum* inoculated alone or in co-inoculations (Spolti and Ponte 2013).

Among more than six hundred isolates identified in Brazil, all *F. graminearum* have the 15ADON genotype and all *F. meridionale* isolates have the NIV genotype (Del Ponte et al. 2015; Kuhnem et al. 2016; Scoz et al. 2009; Astolfi et al. 2012). As expected, all *F. graminearum* isolates produced large amounts of DON and more of their respective acetylate trichothecene, 15ADON. However, detectable trace amounts of 3ADON were also produced either in infected wheat heads or in rice cultures. Similarly, *F. meridionale* isolates produced more NIV and only two of them produced higher levels of DON. Genotyping was consistent with phenotyping for our isolates, even though some other authors have reported some discrepancies with these methods (Mugrabi de Kuppler et al. 2011; Spolti et al. 2014a).

We have reported evidence that *F. meridionale* and *F. graminearum* significantly differ in the overall fitness. Although there is a large degree of intraspecific variation and overlap between species for individual traits, *F. meridionale* and *F. graminearum* are distinct from one another when all the traits are considered together. Traits related to sexual or asexual fertility, such as perithecial/ascospore and macroconidial production, and aggressiveness to wheat heads contributed the most to distinguish isolates of *F. graminearum*. In contrast, aggressiveness on maize silks and mycelial growth highly related to *F. meridionale* strains. Previous studies might have failed to distinguish these two species due to the low number of strains sampled, because the

strain-specific variation in individual traits can mask overall variation between species (Spolti et al. 2012a; Kuhnem Júnior et al. 2013; Nicolli et al. 2015, 2018). Though not statistically significant, there is weak evidence supporting a hypothesis of interaction between species and the host of origin in overall fitness, which should be more deeply explored in future studies.

Several traits were significantly correlated one to another. The highest correlation was observed between FHB severity and DON production in wheat heads. This is not unexpected because DON is well known to be an aggressiveness factor (Bai et al. 2002; Desjardins et al. 1996; Harris et al. 1999; Maier et al. 2006). DON production in rice culture, on the other hand, did not correlate with DON production *in planta*, suggesting that this trait represents the broader toxigenic potential of each isolate (Goswami and Kistler 2005; Walker et al. 2001). NIV production, either *in planta* or *in vitro*, was negatively associated with all the other traits except for mycelial growth.

As already suggested in the first two chapters and elsewhere (Kuhnem et al. 2016), we wish to emphasize once again that detailed surveys of the mycotoxins found in maize in Brazil are desperately needed. It would also be advisable for breeding programs to use the most aggressive and highly adapted isolates representing the fungal population in the region of interest to evaluate resistance in breeding lines. Increasing surveillance is needed to avoid the introduction of host-adapted toxigenic species into other countries, especially in locations where *F. meridionale* is absent, or not found yet, such as the United States and Canada. Likewise, it is critical to adjust the mycotoxin regulations to include NIV as well as DON levels, considering its importance to public health.

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SUPPLEMENTARY MATERIAL

Table S1. Summary information of the working collection of 45 arbitrarily selected isolates representing *Fusarium graminearum* (*n* = 18) and *F. meridionale* (*n* = 27) obtained from naturally infected maize kernels and symptomatic wheat heads in surveys of commercial fields in southern Brazil from 2009 to 2011.

Specie/ Genotype	Id.	Host	Year	Municipality	State
Fgra	188	wheat	2009	Panambi	RS
Fgra	189	wheat	2009	Ijuí	RS
Fgra	190	wheat	2010	Coxilha	RS
Fgra	191	wheat	2007	Cruz Alta	RS
Fgra	192	wheat	2007	Ernestina	RS
Fgra	193	wheat	2010	Tapejara	RS
Fgra	194	wheat	2011	Não-me-Toque	RS
Fgra	195	wheat	2011	Palmeira das Missões	RS
Fgra	196	wheat	2010	Caseiros	RS
Fgra	197	wheat	2010	Coxilha	RS
Fgra	07	wheat	2010	Estação	RS
Fgra	199	wheat	2011	Ijuí	RS
Fgra	15	maize	2011	Bom Jesus	RS
Fgra	205	maize	2011	Vacaria	RS
Fgra	207	maize	2011	Bom Jesus	RS
Fgra	209	maize	2011	Bom Jesus	RS
Fgra	210	maize	2009	Vacaria	RS
Fgra	211	maize	2009	Vacaria	RS
Fmer	01	wheat	2009	Ernestina	RS
Fmer	02	wheat	2009	Coxilha	RS
Fmer	03	wheat	2007	Nonoai	RS
Fmer	04	wheat	2011	Marau	RS

Fmer	05	wheat	2011	Sertão	RS
Fmer	198	wheat	2010	Água Santa	RS
Fmer	06	wheat	2010	Tapejara	RS
Fmer	08	wheat	2009	Santa Bárbara do Sul	RS
Fmer	21	wheat	2007	Nonoai	RS
Fmer	09	maize	2011	Marechal Cândido Rondon	PR
Fmer	10	maize	2011	Ponta Grossa	PR
Fmer	11	maize	2011	Irati	PR
Fmer	12	maize	2011	Casca	RS
Fmer	13	maize	2011	Eldorado do Sul	RS
Fmer	14	maize	2011	Bom Jesus	RS
Fmer	16	maize	2011	Taguaí	SP
Fmer	17	maize	2011	Paranapanema	SP
Fmer	18	maize	2011	Alambari	SP
Fmer	200	maize	2011	Ponta Grossa	PR
Fmer	201	maize	2011	Ponta Grossa	PR
Fmer	202	maize	2011	Ponta Grossa	PR
Fmer	203	maize	2011	Palmeira	PR
Fmer	204	maize	2011	Palmeira	PR
Fmer	206	maize	2011	Vacaria	RS
Fmer	208	maize	2011	Bom Jesus	RS
Fmer	19	maize	2009	Caxias do sul	RS
Fmer	20	maize	2009	Boa Vista das Missões	RS

[a] Species and trichothecene genotype identified using the multilocus genotype method (Ward et al. 2008). Fgra = *F. graminearum* with 15-acetyl-deoxynivalenol genotype, Fmer = *F. meridionale* with nivalenol genotype.
[b] Isolates were obtained either from maize kernels (Kuhnem et al. 2016) or symptomatic wheat heads (Del Ponte et al. 2015) in previous surveys.
[c] Brazilian states: RS = Rio Grande do Sul, PR = Paraná, SP = São Paulo.

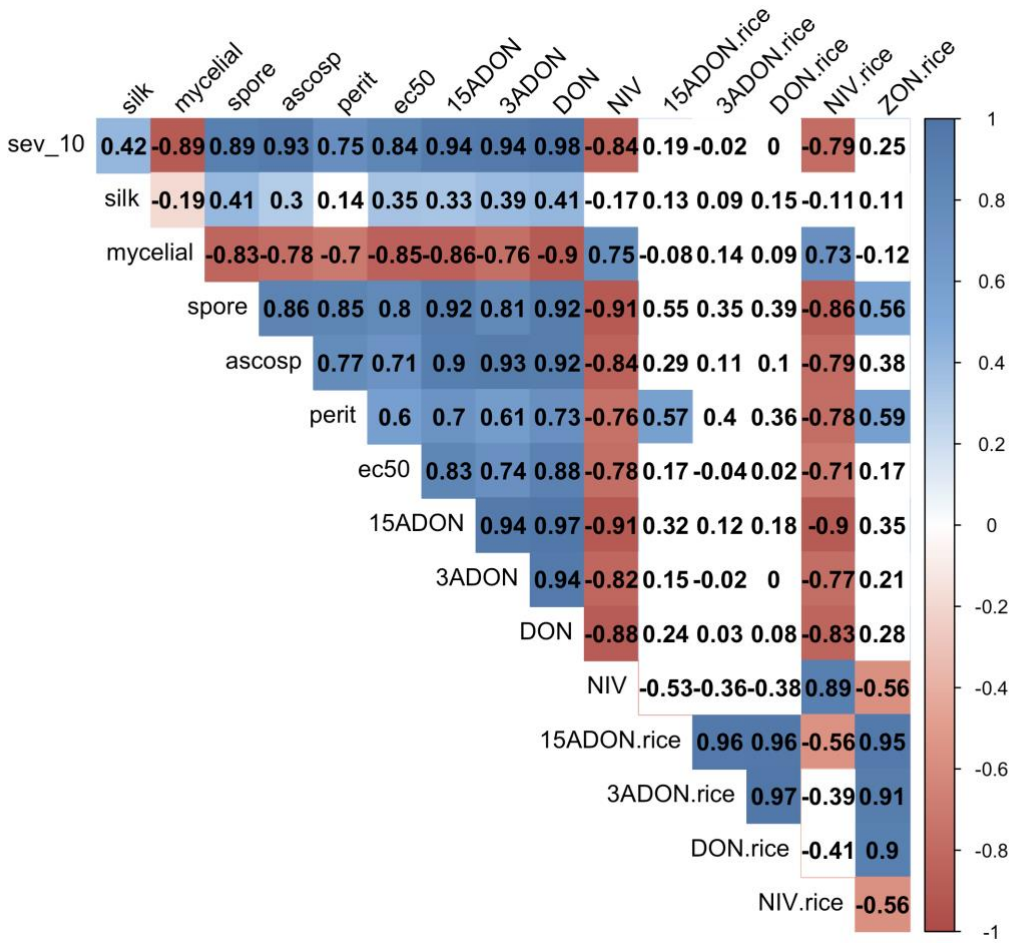

Figure S1. Pearson's correlation coefficients for all pairwise comparisons among saprophytic and pathogenic phenotypic traits disease-variables. Correlation coefficient was calculated using the average each trait per isolate. Negative correlation values are shown in red and positive values in blue. Lighter the color closer the correlation values to zero. Blanks values are not significant at 95% of confidence.

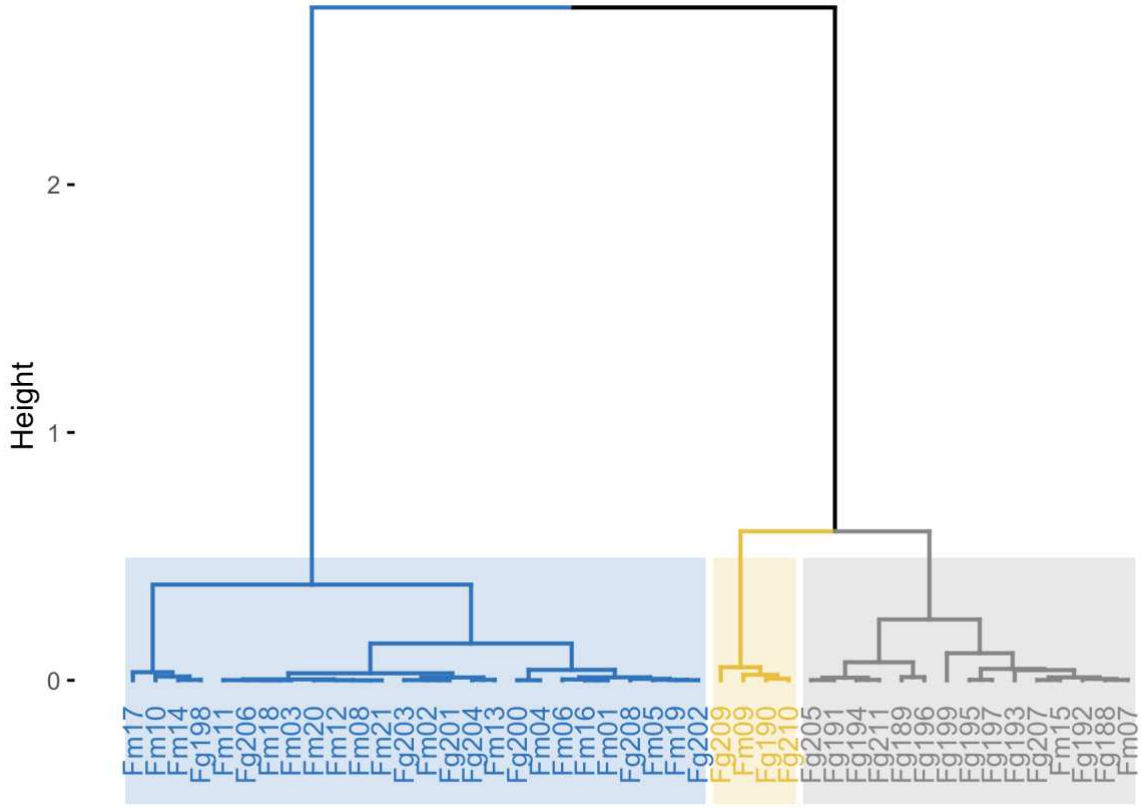

Figure S2. Dendrogram for a sample of 18 *F. graminearum* isolates and 27 *F. meridionale* isolates (n_{maize} = 18; n_{wheat} = 9) obtained from naturally infected maize kernels and symptomatic wheat heads in surveys of commercial fields in southern Brazil from 2009 to 2011 clustered according to their saprophytic and pathogenic traits based on the standardized Euclidean distance and the complete linkage method.

GENERAL CONCLUSIONS

Article 1:

F. meridionale is more aggressive than *F. graminearum* and out-competes the latter when later infecting the ears.

GER severity and NIV production by the more aggressive species *F. meridionale* were both reduced if *F. graminearum* was inoculated after the former.

NIV was produced by *F. meridionale* strains inoculated onto maize ears.

Article 2:

F. meridionale isolates obtained from maize were more aggressive on average on maize ears than *F. graminearum,* confirming the results from chapter 1 that included fewer strains, and suggesting that there is a potential adaptive advantage towards this host tissue.

F. graminearum was more aggressive on maize stalks than *F. meridionale,* with no effect of host of origin in GSR severity.

NIV was the main mycotoxin produced by *F. meridionale* and DON was prevalent in *F. graminearum*-inoculated maize ears in Brazil.

Article 3:

F. graminearum and *F. meridionale* isolates could be grouped together based on their overall reproductive and pathogenic fitness.

There is evidence that the host of origin played an important role in structuring *F. graminearum* and *F. meridionale* population infecting both maize and wheat in southern Brazil.

F. graminearum was confirmed to be more aggressive on wheat and more fit regarding its sexual or asexual reproduction *in vitro*.