***Fusarium meridionale* is more aggressive and competitive than *F. graminearum* as a causal agent of Gibberella Ear Rot of maize in Brazil**

Franklin J. Machado^{1,2*}, Paulo R. Kuhnem^{3,6*}, Ricardo T. Casa⁴, Nicole McMaster⁵, David G. Schmale III⁵, Lisa J. Vaillancourt², Emerson M. Del Ponte¹

¹ Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa MG, Brazil; ² Department of Plant Pathology, University of Kentucky, Lexington KY, United States; ³ Programa de Pós-graduação em Fitotecnia, Universidade Federal do Rio Grande do Sul, Porto Alegre RS, Brazil; ⁴ Universidade do Estado de Santa Catarina, Lages SC, Brazil; ⁵ School of Plant and Environmental Sciences, Virginia Tech, Blacksburg VA, United States; ⁶ Current address: Biotrigo Genética Ltda, Passo Fundo RS, Brazil

* These authors contributed equally to this work

Authors for correspondence: Emerson M. Del Ponte, e-mail: delponte@ufv.br; Lisa J. Vaillancourt, e mail: vaillan@uky.edu

Abstract

In Brazil, Gibberella ear rot (GER) of maize is caused mainly by *Fusarium meridionale* (*Fmer*), while *F. graminearum* (*Fgra*) is a minor contributor. To test the hypothesis that *Fmer* is more aggressive than *Fgra* on maize, three experiments were conducted in the south (subtropical summer), and one in the central-south (tropical winter) of Brazil, totaling seven conditions (year × location × hybrid). Treatments consisted of *Fgra* or *Fmer* (two isolates of each) inoculated once four days after silk; alternately inoculated (*Fgra*□*Fmer* or *Fmer*□*Fgra*) six days apart; and (in central-south only) sequentially inoculated without alternating (*Fmer*□*Fmer* or *Fgra*□*Fgra*). Overall GER severity was higher in the south (37.0%) compared with the central-south (16.9%), likely due to the higher summer temperatures (20 to 25°C). In the south, severity was highest in *Fmer* treatments (67.8%); followed by *Fmer*□*Fgra* (41.1%); then *Fgra*□*Fmer* (19.4%);

and lowest in *Fgra* (2.1%), suggesting an antagonistic relationship. In the central-south during winter (15 to 20°C), severity was generally higher (7.3 to 11.6%) in sequential inoculation treatments than when either species was inoculated alone. Only nivalenol (NIV) or deoxynivalenol (DON) were detected when *Fmer* or *Fgra*, respectively, were inoculated singly and sequentially with no alternation. Both toxins were found in grains harvested from the *Fmer*∅*Fgra* treatment, while only NIV was found in kernels from the *Fgra*∅*Fmer* treatment, suggesting that *Fmer* was more competitive than *Fgra* in co-inoculations. The dominance of *Fmer* as a cause of GER in Brazil may be due in part to its higher aggressiveness and competitiveness than *Fgra*.

KEYWORDS:

Zea mays, *Fusarium graminearum* species complex, nivalenol.

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 2003b). FGSC species 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 reportedly favored by increased levels of moisture around silking, followed by moderate

temperatures and high rainfall during the maturation period (Munkvold 2003b; Sutton 1982).

Both FHB in wheat and barley and GER in maize reduce yield (Duffeck et al. 2019; Munkvold 2003a), but the presence of mycotoxins that accumulate in the kernels and downgrade product value is also a major concern (Munkvold et al. 2019). FGSC species produce several mycotoxins: the most important ones belong to the B-trichothecene group, including nivalenol (NIV), and deoxynivalenol (DON) and its acetylated derivatives 3ADON and 15ADON (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, zearalenone (ZEA) and fumonisins (B1 and B2) are the only mycotoxins that are currently regulated for maize (ANVISA 2011; 2017).

Management of GER aims to reduce infection by toxigenic fungi in order to increase yield and suppress mycotoxin contamination (Munkvold 2003a). The most effective control for GER is the use of host genetic resistance. However, breeding for resistance is complicated by the diversity of *Fusarium* species that can cause GER (Mesterházy et al. 2012; Munkvold 2003a). 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 (*Fmer*), the most common FGSC species isolated from maize (Kuhnem et al. 2016), 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). Nonetheless, trichothecene mycotoxins typically produced by GER pathogens have recently been found contaminating commercial maize grain in Brazil (Oliveira et al. 2017).

Regional shifts in host dominance among FGSC species causing FHB in wheat and GER in maize have been reported, including in South American wheat-growing regions (Castañares et al. 2016; Del Ponte et al. 2015; Gomes et al. 2015; Kuhnem et al. 2016; Sampietro et al. 2011). For example, *F. graminearum* (*Fgra*) of the DON/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 North America, GER is also caused mainly by *Fgra* of the DON/15ADON type, according to a survey conducted in Ontario, Canada (Burlakoti et al. 2017). *Fusarium boothii*, another DON/15ADON-producing species, was reported as the primary cause of GER in South Africa (Boutigny et al. 2011). In Brazil, *Fmer*, a NIV-producing species, is the dominant FGSC species in maize, and is also increasing in importance in wheat in regions where maize is a major crop (Del Ponte et al. 2015; Pereira et al. 2021). Other NIV-producing FGSC species (*F. asiaticum*, *F. cortaderiae* and *F. austroamericanum*) and also some non-NIV, non-FGSC species (*F. culmorum*, *F. cerealis* and *F. poae*) have been found in association with GER in maize in other regions of the world

(Basler 2016; Desjardins and Proctor 2011; Kuhnem et al. 2016; Lee et al. 2012; Ndoye et al. 2012; Zhang et al. 2016).

The toxin profile of different FGSC species has been considered to influence pathogenesis (Ward et al. 2002). A recent survey of mycotoxins contaminating maize grains in southern Brazil was consistent with previous work suggesting that *Fmer* was the primary maize pathogen. Authors of that study reported the presence of NIV, which is produced by *Fmer*, and rarely by *Fgra*, in more samples (76%) than DON (48%), though levels for both were lower than the Brazilian limits of contamination for DON (Oliveira et al. 2017). While DON has been confirmed as an aggressiveness factor that facilitates fungus spread within wheat heads and, to a lesser extent, in 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 maize ears remains unclear (Maier et al. 2006). There are very few studies that have investigated differential aggressiveness among members of the FGSC with different toxin profiles that cause GER.

Multiple members of the FGSC and of other *Fusarium* species complexes can co-occur in the same field, or even on the same maize ear (Logrieco et al. 2002; Oldenburg et al. 2017; Picot et al. 2012). Previous studies have investigated interactions between *Fgra* and *Fusarium* spp. in maize ears (Picot et al. 2012; Reid et al. 1999); however the effects of any interspecific interaction between FGSC members on GER development are unknown. In contrast, intra- and inter-species competition interactions, including antagonistic intrapopulation interactions, have been demonstrated for FHB pathogens on wheat heads and in

vitro (Vaughan et al. 2020; Walkowiak et al. 2015; Wagacha et al. 2012; Miedaner et al. 2004; Siou et al. 2015; Xu et al. 2007).

We hypothesized that (1) strains of *Fmer* are more aggressive and competitive and (2) produce significantly greater levels of NIV trichothecenes in maize ears, when compared with *Fgra* strains. Isolates were inoculated singly, and they were also inoculated alternately and sequentially (six days apart), to study interspecific interactions. We conducted experiments at three different locations with contrasting weather patterns, mainly temperature. The results of this study could help to explain why *Fmer* dominates as the GER pathogen of maize in Brazil.

Material and Methods

Fungal isolates and preparation of inoculum

Two *Fgra* (DON/15ADON-producing) isolates and two *Fmer* (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 demonstrated the dominance of *Fmer* 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 hemocytometer. Spore

suspensions of the two isolates for each species were diluted in sterile water and then mixed in a 1:1 ratio (v/v) to achieve the desired concentrations (5×10^5 macroconidia/ml) (Reid et al. 1995).

Treatments and inoculation procedures

Each species was inoculated individually, at four days after silking (Reid et al. 2002). Two other treatments consisted of alternate inoculations of the two species (the second six days after the first), for a total of four pathogen inoculation treatments [*Fgra*; *Fmer*; *Fgra*□*Fmer*; *Fmer*□*Fgra*]. In one location only, the two species were also inoculated sequentially, six days apart (*Fgra*□*Fgra*; *Fmer*□*Fmer*). A mock inoculation (sterile distilled water) was included as a negative control (Table 1). Plants were individually inoculated by using a syringe with an obtuse needle to inject the suspensions (2 ml of a 5×10^5 macroconidia/ml solution) into the silk channel of the primary ear (Anderson et al. 2016). Inoculations were followed by three consecutive days of irrigation.

Experimental field design

The inoculation treatments were evaluated under field conditions on ear-rot susceptible commercial hybrids (P30F53 HR®, STATUS TL®, and RB9004 PRO2®) in two regions in Brazil with distinct climatic patterns. The field trials were arranged in a randomized complete block design with four replications, and each of the five inoculation treatments was randomly assigned to the experimental units.

Table 1. Single and sequential inoculation of *Fusarium graminearum* (Fgra) and *Fusarium meridionale* (Fmer) in the early stages of silk development of maize ears.

Treatment ^a	Silking + 4 days	Silking + 10 days
Fgra ^b	Fgra	-
Fmer ^c	Fmer	-
Fgra□Fmer	Fgra	Fmer
Fmer□Fgra	Fmer	Fgra
Fgra□Fgra ^d	Fgra	Fgra
Fmer□Fmer ^e	Fmer	Fmer
Mock inoculations	Water	Water

^aAll 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). Isolate codes: Fmer: 09Mi034 and 09Mi085; Fgra: 09Mi010 and 09Mi011

^bMix of two *F. graminearum* (15ADON) isolates.

^cMix of two *F. meridionale* (NIV) isolates.

^{d,e}Treatments evaluated only in the Viçosa/MG trial.

In the south subtropical region, the experiments were conducted 400 km apart in two municipalities in each of two Brazilian states during the 2011/2012 and 2012/2013 maize growing seasons (Table 2). The hybrids P30F53 HR® and STATUS TL® were compared in these experiments. The inoculation treatments were randomly assigned to one row (6-m-long row with 50 cm between rows) in each replicate (block). The ten central plants (comprising an experimental unit) of each row were inoculated. Inoculated rows were bordered by uninoculated plants of the same variety. Fertilization and weed control were performed following local recommendations. The plots were sprinkle-irrigated as needed.

In the tropical south-central region of Brazil, an experiment was conducted at the experimental station of the Universidade Federal de Viçosa, during the 2017 winter growing season (Table 2). Seeds of the maize hybrid RB9004 PRO2® were sown in April. The experimental unit consisted of a 5-m-long row (60 cm between rows) with 18 plants. The central ten plants in each row were inoculated. Inoculated rows were bordered by uninoculated plants of the same variety. The entire field was fertilized following chemical soil analyses, and sprinkle-irrigated as needed.

In each of the three sites, daily summaries of relative humidity and temperature were measured at meteorological stations located within 500 m of distance to the experimental (Figure S6). We did not collect rainfall data, as the trials received irrigation.

Table 2. Summary information of the three trials conducted in the south (subtropical summer) and one conducted in the central-south (tropical winter) of Brazil during 2012, 2013 and 2017 harvest seasons.

Location ^a	Latitude	Longitude	Alt (m)	Hybrid	Planting date	Inoculation date	Harvest date
Lages SC	27°47'34" S	50°18'15" W	940	P30F53 HR®; STATUS TL®	14 Nov 11	17 to 20 Jan 12	9 Mar 12
Eldorado RS	30°05'31" S	51°40'19" W	046	P30F53 HR®; STATUS TL®	3 Oct 11	2 to 3 Jan 12	9 Mar 12
Eldorado RS	30°05'31" S	51°40'19" W	046	P30F53 HR®; STATUS TL®	1 Oct 12	26 Dec 12	20 Feb 13
Viçosa MG	20°44'44" S	42°50'59" W	661	RB9004 PRO2®	25 Apr 17	07 Ago 17	25 Set 17

^aMunicipality and State of Brazil where the trials were carried out. South region (subtropical summer): SC = Santa Catarina and RS = Rio Grande do Sul states. Central-south (tropical winter): MG = Minas Gerais state.

Disease and mycotoxin assessment

When grain moisture content reached 22% for the southern subtropical trials, or physiological maturity stage for the south-central tropical trial, the ten inoculated ears for each treatment were handpicked and husked. The GER severity on each ear was rated as the percentage area of the ear covered with fungal mycelia (Reid et al. 2002; Reid and Zhu 2005).

Trichothecenes and ZEA mycotoxins were measured in maize kernels from the Viçosa trials. 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 Schmale Lab at Virginia Tech in Blacksburg (Virginia). The amount of DON and each of its acetylated forms (15ADON and 3ADON), NIV, and ZEA were quantified using gas chromatography–mass spectrometry (Fuentes et al. 2005; Mirocha et al. 1998).

Data analysis

A single multilevel (hierarchical or linear mixed) model was fitted to the GER severity data from the southern trials (Eldorado and Lages). The main reasons for using the multilevel model included 1) observational units could not be assumed to be independent and 2) inferences can be made to a population of

groups because groups in the sample are treated as a random sample from a population of groups.

In this modelling framework, multiple random-effects capture how observations deviate within a cluster, and how each cluster deviates from the overall group. A three-level model was then fitted. The effects of interest (the inoculation treatments) were nested by study design, i.e. GER severity was measured on individual maize ears (level 1), nested within the hybrids (level 2), and hybrids were nested within trials (level 3). A model of nested random effects (cultivar within trial) was specified because cultivar was reused across the trials.

For the Viçosa trial, a linear mixed model was fitted to the log-transformed ($\log + 0.5$) GER severity data. Inoculation treatment and block were treated as fixed effects, and individual ears within blocks as random effects.

The simple linear and the multilevel models were fitted using the `lm` and `lmer` (`lme4` package) functions in R (R Core Team 2020). Different transformations (angular, log and logit) were tested and chosen according to residual analysis. The model residuals were diagnosed using the 'DHARMA' package, which uses a simulation-based approach to create readily interpretable scaled (quantile) residuals for fitted (generalized) linear mixed models (Hartig and Lohse 2020). This package provided *P*-values for three tests of model quality (normality of residuals, overdispersion and outlier). A type-III analysis-of-variance table was obtained using the `Anova` function of the `car` package (Fox and Weisberg 2019). Post hoc analysis was performed using the `emmeans` package to obtain estimated marginal means and respective 95%

confidence intervals, and comparisons thereof via pairwise P-value plots (Lenth et al. 2020).

Results

Southern trials

GER symptoms were not observed in the non-inoculated controls, suggesting the absence of background inoculum. Symptoms of GER were observed in almost all ($n = 204$) inoculated ears that were visually scored. Visual symptoms were not present in 32/204 (15.6%) of the inoculated ears; most of the zeros (60%) were found in the *Fgra* inoculation treatments across all six environments (Fig. 1). There was a trend of increasing GER severity when *Fmer* was inoculated after *Fgra* or alone. The *Fmer* treatment, in particular, produced the highest median of GER severity in almost all trials. When *Fmer* was followed by *Fgra* inoculation, GER severity tended to decrease. The variability in the severity measures was quite high, and tended to increase with an increase in severity; individual values ranged from zero to 100% in the same treatment (Fig. 1).

The simulation-based residual analysis did not detect any issue related to distribution, dispersion and outliers with the selected model specification (Fig. S1). The null hypothesis of the effect of inoculation treatment was rejected ($\chi^2 = 67.8$, $P < 0.0001$). The estimated marginal means (EMMs) of the fitted model ranged from 0.02 (2%) for *Fgra* inoculated alone to 0.41 (41%) for *Fmer* inoculated alone. Plots for the pairwise Tukey-adjusted P-value (Fig. 2A) and the EMMs and

95% confidence interval (Fig. 2B) show that *Fmer*, and *Fmer* followed by *Fgra*, produced statistically similar values of GER severity.

Figure 1. Distribution of Gibberella ear rot (GER) visual severity estimates on individual plants (ear, dot) inoculated with in-field inoculation treatments composed of single-time inoculation of either *Fusarium graminearum* (*Fgra*) or *F. meridionale* (*Fmer*) or sequential (second inoculation at 6 days after the first) alternated inoculation of the same species in different order (*Fgra*→*Fmer* or *Fmer*→*Fgra*). The six environments (plots) correspond to a combination of location × year × hybrid (EL = Eldorado, RS, Brazil) or LG (Lages, SC, Brazil), harvest seasons (2012 or 2013), and maize hybrids (P30F53 HR® and STATUS TL®).

Viçosa trial

Similar to the southern trials, no GER symptoms were found in the non-inoculated controls. GER severity values ranged from zero (4% of all ears) to 100% (0.5% of all ears). The distribution of the severity values was right-skewed with an overall mean (median) of 16.9% (8%). The log-transformation helped to normalize the distribution of the model residuals (Fig. 3A-B) and stabilize variances (box-plot in Fig. S2). Similar to the southern experiments, there was a considerable variation in the GER severity values within the same block (Fig. 3C).

Figure 2. **A)** Pairwise P-value plot depicting the estimated marginal means (EMMs) (colored proportion values) by a multilevel model fitted for *Gibberella* ear rot (GER) severity (proportion visible area affected) on maize grown at six location $\times$ year $\times$ hybrid combinations, and the respective probability values for each pairwise comparison. **B)** EMMs for percent GER values and respective 95% confidence intervals.

The simulation-based residual analysis of the fit of the linear model did not show any issues with the chosen transformation ($\log+0.5$) (Fig. S3). The null hypothesis of the effect of inoculation treatment was rejected ($F = 8.81$, $P < 0.0001$). The estimated marginal means (EMMs) of the fitted model ranged from 2.4% for *Fgra* inoculated alone to 11.7% for *Fmer* inoculated sequentially.

Figure 3. Histograms for the distribution of *Gibberella* ear rot (GER) visual severity (A) and log-transformed GER severity data (B) for normalizing model residuals. The visual GER severity was estimated on individual plants (each dot represents a single ear), grouped in blocks (6 to 10 ears in a box-plot) inoculated with treatments consisting of a single inoculation of either *Fusarium graminearum* (*Fgra*) or *F. meridionale* (*Fmer*); sequential (second 6 days after the first) inoculation of the same species (*Fgra*→*Fgra* or *Fmer*→*Fmer*); or sequential alternated inoculation of both species (*Fgra*→*Fmer* or *Fmer*→*Fgra*) C). The RB9004 PRO2[®] maize hybrid was cultivated during the 2017 growing season in Viçosa, Minas Gerais state (MG).

The pairwise Tukey-adjusted P-values (Fig. 4A) plot shows a range of values varying from 0.001 to 1, with three comparisons around the 5% significance level. The appraisal of the P-value plot and the plot with the back-transformed means and 95% confidence interval (Fig. 4B), suggest statistically similar severity for sequential inoculation of the same species (*Fmer* or *Fgra*) or when *Fmer* was inoculated after *Fgra*. The lowest, statistically similar ($P = 0.056$) GER severity values, were estimated for the two single-time inoculation of either species (Fig. 4A).

Figure 4. A) Pairwise P-value plot (multiple comparison) depicting the estimates of marginal means (EMMs) by a multilevel model fitted for log-transformed values (shown in color next to the treatment code) of Gibberella ear rot (GER) severity (percent affected area) on maize (RB9004 PRO2®) grown during the 2017 growing season in Viçosa, Minas Gerais state (MG, Brazil). **B)** Back-transformed EMMs (percent severity) values and respective 95% confidence intervals. Treatments were: single-time inoculation of either *Fusarium graminearum* (*Fgra*) or *F. meridionale* (*Fmer*); sequential (the second 6 days after the first) inoculation of the same species (*Fgra*→*Fgra* or *Fmer*→*Fmer*);

and sequential alternated inoculation in different order (*Fgra*□*Fmer* or *Fmer*□*Fgra*).

Trichothecene levels in kernels from *Fmer* and *Fgra* inoculated singly ranged from 0.50 to 2.05 µg/g (ppm), and from 0.6 to 21.25 µg/g for the two sequential inoculations of either species respectively (Fig. 5). For the sequential alternated inoculation treatments, DON and NIV levels ranged from 0.35 to 3.15 µg/g. DON was detected in only one sample of *Fmer*□*Fgra* at similar levels with NIV and ZEA (0.60 µg/g, 0.70 µg/g and 0.40 µg/g, respectively). Only NIV was detected in kernels from ears inoculated with *Fmer* alone, whereas DON was the only mycotoxin detected in kernels from the *Fgra* treatment. In contrast, NIV was abundant in kernels from ears of the *Fgra*□*Fmer* treatment. In the *Fmer*□*Fgra* treatment NIV, DON, and a smaller amount of ZEA were all detected (Fig. 5). For the sequential inoculation of *Fgra*, DON (7.96 µg/g) was detected in all kernel samples whereas 15ADON (1.08 µg/g) and 3ADON (0.48 µg/g) were detected in half of them (Fig. 5). Finally, five times more NIV was detected in kernels from ears inoculated sequentially with *Fmer* (6.15 µg/g), compared to the single-time inoculation treatment (1.25 µg/g). There was a clear association between NIV production and GER severity (Fig. 4B and Fig. 5).

Seasonal temperature and relative humidity

The daily temperature was variable across the four experiments during the 37-day period relative to the inoculation. In the subtropical summer trials, where the inoculations and the development of the disease occurred during the summer (Dec to Jan), the mean temperatures were generally higher than in the tropical winter, ranging mostly between 20°C and 26°C in the two low-elevation Eldorado trials. In the high-elevation Lages site, the minimum temperatures were markedly lower than in the Eldorado (Fig. 6).

Figure 5. Mean production of deoxynivalenol (DON) and its acetylated forms [15-acetyl-deoxynivalenol (15ADON) and 3ADON], nivalenol (NIV) and zearalenone (ZEA) 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 (bulk sample of kernels from 10 inoculated ears) had detectable mycotoxins. Measurements in the missing blocks were under the limit of detection ($< 0.25 \mu\text{g/g}$).

The relative humidity was slightly lower in 2013 than in 2012 in Eldorado, and inversely related with temperature (Fig. 6). In the Viçosa trial, conducted during a tropical winter, the mean temperature was generally between 15 and 20°C. The minimum temperatures were the lowest across all the experiments, ranging from 8 to 11°C. The relative humidity was also the lowest (Fig. S4), although the plots received supplemental irrigation.

Figure 6. Daily values temperature (minimum, mean and maximum) and relative humidity recorded at the nearest meteorological station during the maize inoculation experiment conducted at four experimental sites in southern (Eldorado and Lages) and central-eastern (Viçosa) Brazil during three seasons.

Discussion

New information is needed to understand why different phylogenetic species within the FGSC are dominant among isolations from different cereals, or from the same cereal in different regions of the world (Boutigny et al. 2011; Carter et al. 2000; Castañares et al. 2016; Del Ponte et al. 2015; Gomes et al. 2015; Kuhnem et al. 2016; Sampietro et al. 2011; Umpiérrez-Failache et al. 2013). Overall, *Fgra* is the most common cause of FHB in wheat and barley worldwide (Del Ponte et al. 2015; Kelly and Ward 2018), and is the most frequent cause of GER and GSR in North America (Kuhnem et al. 2015). However in South America, including in northwestern Argentina and in the south of Brazil, as well as in Nepal, *Fmer* is more prevalent as the causal agent of GER and GSR (Desjardins and Proctor 2011; Kuhnem et al. 2016; Sampietro et al. 2011).

Previous reports have shown that aggressiveness-related traits contribute to shaping the profile of regional populations that co-exist and are shared among different cereal crops. The role of differential aggressiveness has been studied previously in wheat involving a larger set of FGSC species (Goswami and Kistler 2005; Nicolli et al. 2015, 2018; Spolti et al. 2012). These experiments showed that *Fgra*, which dominates on wheat, was also more aggressive than these other species on wheat. However, there are relatively few experiments looking at this question in maize. In one example, *F. boothi*, a DON/15-ADON producer that was the most prevalent species in field surveys in South Africa, was more aggressive and produced higher levels of mycotoxins compared with

DON-producing wheat isolates of *Fgra* and NIV-producing *Fmer* (Beukes et al. 2018).

To test the hypothesis that *Fmer* is more aggressive than *Fgra* on maize, three experiments were conducted in the south (subtropical summer), and one in the central-south (tropical winter) of Brazil, totaling seven conditions (year $\times$ location $\times$ hybrid). To our knowledge, this is the first detailed study to examine aggressiveness, competitiveness and mycotoxin production by *Fmer* and *Fgra* on maize ears in field experiments in Brazil. It extends previous work that compared inoculation methods and hybrid resistance using a mixture of *Fmer* isolates (Nerbass et al. 2015; 2016). Using mixtures of two representative strains of each of the two species, our results supported our hypothesis that enhanced aggressiveness and competitiveness of *Fmer* is an important contributor to its dominance as the major GER pathogen in the country (Kuhnem et al. 2016). This conclusion does not preclude roles for other aspects of fitness and competitiveness in this observed dominance: for example *Fmer* may produce more ascospores or conidia, or it may survive better as a saprophyte on crop debris, or on alternative hosts. Further experiments will be needed to investigate the potential impact of these factors.

In our experiments conducted during the summer in the south of the country, we found that GER severity was, on average, twice as high when *Fmer* was inoculated alone, compared with solo inoculations of *Fgra*. During the period following the inoculations, mean temperature fluctuated between 20 and 25°C, which was higher than the mean temperatures the crop experienced during the tropical winter in the Viçosa trial, where the relative humidity was

also lower than in the south trials. The difference in GER severity between the species was reduced, but still significant, in the tropical winter trial where the inoculated plants experienced temperatures of around 5°C right after the inoculation. Two sequential inoculations, six days apart, performed only in the tropical winter trial, produced higher levels of disease for both species, with *Fmer* producing more disease than *Fgra*. Thus, results in all locations supported the hypothesis that *Fmer* is more aggressive on maize.

The overall increase in disease severity in the southern experiments is in agreement with several previous studies that, contrary to suggestions that GER is a disease of cooler weather (Munkvold 2003b), also reported increased severity or fungal growth under wetter and warmer temperatures (20 to 30°C) (Sutton 1982; Dalla Lana et al. 2020). A previous study involving two strains of each species reported that *Fgra* grew faster than *Fmer* at 25°C and 30°C, whereas growth rates were similar at 10, 15 or 20°C (Spolti et al. 2012). However, a more recent study using 20 strains from each species showed a wide variation in growth rates among isolates of the same species at both at 15 and 25°C (Kuhnem et al. 2016). Thus, the response to temperature is isolate-specific rather than species-specific, implying that the increased disease severity observed in the southern experiments is not due to a direct effect of temperature on the growth of *Fgra* versus *Fmer*. Further experiments are needed to explore whether the maize hybrid played a role in the disease outcomes in the different locations, as had been reported in other studies (Nerbass et al. 2016).

Sequential alternate co-inoculation of maize ears with *Fgra* and *Fmer* suggested an antagonistic behavior, as this treatment generally produced lower disease levels than *Fmer* inoculated alone once, or sequentially twice. Results of mycotoxin assays suggested that *Fmer* was more competitive in co-inoculations if it was inoculated after, versus before *Fgra*. Both disease severity and NIV production were reduced when *Fgra* was inoculated into the same ear six days later, versus when *Fmer* was inoculated alone or sequentially twice. This suggests that growth of *Fmer* was suppressed by the growth of *Fgra*. In contrast, when *Fgra* was the first to colonize the inoculated site, neither DON nor ZEA was detected, suggesting that growth of *Fgra* was prevented by subsequent inoculation of *Fmer*. However, if *Fgra* was inoculated sequentially twice, it produced much more DON and higher disease severities than when it was followed by *Fmer*. Interestingly, in this case GER severity was higher than when either species was inoculated alone, or when *Fmer* was inoculated first. Damage caused by the initial inoculation with *Fgra* may have had an effect similar to wounding caused by lepidopteran larvae, acting as a breach to facilitate colonization by a second inoculation with *Fmer* or *Fgra* (Picot et al. 2012). The higher overall toxin concentration in ears infected by *Fgra* versus *Fmer* may be due to a generally higher amount of toxin produced by *Fgra*, as reported in previous comparative studies (Nicolli et al. 2015; Goswami and Kistler 2005; Tóth et al. 2005). Although the mycotoxin results represent only a single experiment, they suggest that interspecies interactions can have significant qualitative and quantitative effects on mycotoxin contamination in maize ears infected by GER. Further research is needed to better understand the role of these interactions in

co-inoculations. In addition, it would be instructive to design and test specific markers to measure DNA content as a surrogate for biomass of each species (Vegi et al. 2011; Demeke et al. 2010), since they cannot be distinguished based on symptoms alone.

Our results suggest that breeders should consider carefully the inoculum they use during screening for resistance. Inoculum should represent strains and species that are dominant in the region where the hybrids will be deployed (Nerbass et al. 2016). It will also be important to test the significance of the species x hybrid interaction using a larger number of host genotypes. Data on this aspect in several wheat genotypes inoculated with *Fgra* or *Fmer*, where no significant species-cultivar interaction was found, was recently published (Mendes et al. 2018).

This study provides the first direct evidence of the ability of *Fmer* causing GER in Brazil to produce NIV in maize ears. This mycotoxin was recently reported to be the most frequent in surveys of commercial maize in Brazil (Oliveira et al. 2017). This finding confirms the importance of this pathogen in GER epidemics, and the need to include analysis of NIV mycotoxin, which is currently not a target for regulation, and lacking in routine mycotoxin surveys of Brazilian maize.

Acknowledgements

F. J. Machado thanks the Programa de Pós-graduação em Fitopatologia (UFV), CNPq-Conselho Nacional de desenvolvimento Científico e Tecnológico for

providing a graduate scholarship and CAPES-Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for providing a sandwich fellowship. P. Kuhnem thanks the Programa de Pós-graduação em Fitotecnia (UFRGS) and CNPq for providing a graduate scholarship. Thanks are given to CNPq for providing a research fellowship to E. M. Del Ponte and R. T. Casa. This work was partially supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Multistate Project #1008664.

Data availability statement

Conflict of interest

Author contribution

FJM and PRK conceived and conducted the field experiments, analysed the data and wrote the manuscript. RTC, DGSIII and NM conducted experiments and reviewed the manuscript. LJV and EMD conceived, wrote and submitted the manuscript.

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SUPPLEMENTARY MATERIAL

FIGURES

Figure S1. Visual outputs of the residual analysis by `plot.DHARMA()` function of the DHARMA R package for the model fitted to the six environments in the South trial. The `plotQQunif()` function (left) to detect overall deviations from the expected distribution with added P-values of tests for correct distribution (KS test), dispersion and outliers. The `plotResiduals()` function produces a plot (right) of the residuals against the predicted value (or alternatively, other variable).

Figure S2. Distribution of log-transformed *Gibberella* ear rot (GER) visual severity estimates on individual plants (ear, dot) inoculated with inoculation treatments composed of single-time inoculation of either *Fusarium graminearum* (Fgra) or *F. meridionale* (Fmer) or sequential (second 6 days after the first) inoculation of the same species (Fgra2x or Fmer2x) or sequential alternated inoculation of the same species in different order (Fgra → Fmer or Fmer → Fgra). The RB9004 PRO2[®] maize hybrid was cultivated during the 2017 growing season in Viçosa, Minas Gerais state (MG).

Figure S3. Visual outputs of the residual analysis by `plot.DHARMA()` function of the DHARMA R package for the model fitted to GER data from the Viçosa trial. The `plotQQunif()` function (left) to detect overall deviations from the expected distribution with added P-values of tests for correct distribution (KS test), dispersion and outliers. The `plotResiduals()` function produces a plot (right) of the residuals against the predicted value (or alternatively, other variable).

Figure S4. Violin-plots and box-plots (median and upper-lower quartile) for the distribution of temperature (minimum, mean and maximum) and relative humidity recorded at the nearest meteorological station during a period of around a month after inoculation of maize. Experiments were conducted at four environments (site x year) in southern (Eldorado and Lages, 2012 and 2013 harvest year) and central-eastern (Viçosa, 2017 harvest year) Brazil.