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

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ABSTRACT

Gibberella ear (GER) and stalk rot (GSR) of maize in Brazil are caused mainly by *Fusarium meridionale*, while *F. graminearum* is the more common cause of Fusarium Head Blight (FHB) on wheat. Our previous study of two maize isolates of each species suggested that this pattern of dominance may be due to greater aggressiveness and competitiveness of *F. meridionale* on maize. Here we have evaluated pathogenicity and toxigenicity of 16 isolates of *F. graminearum* and 24 isolates of *F. meridionale* recovered from both wheat and maize. Strains were individually inoculated into ears of four maize hybrids in field trials. GER severity varied significantly among isolates within each species. Although ranges overlapped, average GER severity induced by *F. meridionale* (13.9%) was two times higher than *F. graminearum* (7.2%) for isolates obtained from maize, but similar for those isolated from wheat (11.8 and 10.6% respectively). In contrast, severity of GSR was slightly numerically higher for *F. graminearum* (18.4%) versus *F. meridionale* (16.1%), with no effect of host of origin. Deoxynivalenol (DON) and its acetylated form 15ADON were the main mycotoxins produced by *F. graminearum* (7/16 strains), while nivalenol (NIV) was the only toxin produced by *F. meridionale* (17/24 strains). Six isolates of *F. graminearum* also produced zearalenone. Results confirm that *F. meridionale* from maize is, on average, more aggressive on maize, but suggest greater complexity related to diversity among individual isolates within each species. Further studies involving other components of the disease cycle may help to more fully explain the observed pattern of host dominance.

Key-words: *Zea mays*, Gibberella ear rot, Gibberella stalk rot.

INTRODUCTION

Members of the *Fusarium graminearum* species complex (FGSC) are Ascomycete fungi that cause Gibberella ear rot (GER) and stalk rot (GSR) on maize as well as Fusarium head blight (FHB) in small grains. These diseases cause significant reductions in 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 (Bai et al. 2002; Desjardins et al. 1996; Maier et al. 2006). DON also reportedly contributes to GER severity (Harris et al. 1999). 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 where FHB is of common occurrence leading to crop losses (Del Ponte et al. 2009; Duffeck et al. 2020). Ear and stalk rots caused by FGSC are increasing in importance as the application of improved technologies and agronomic practices that include 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;

2017), 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 complex 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). It is recommended that breeders should include adapted strains of regionally dominant species when screening for host resistance (Machado et al., 2021).

The hypothesis that FGSC pathogen species differ in their ability to survive and compete in 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). In a previous study, we reported that *F. meridionale* was more aggressive and competitive than *F. graminearum* when it was used to inoculate or co-inoculate maize ears (Machado et al., 2021). However, the small sample size (two isolates of each species, from only maize); the combining of the isolates, thus masking any intraspecific differences; and the focus on only one stage of the disease cycle (colonization) and one organ (ears), limit our ability to conclude that these differences have a major role in shaping FGSC composition in the field. In the current study, our main objective was to characterize aggressiveness on maize ears

and stalks, and mycotoxin production in maize ears, by a larger group of characterized strains of *F. graminearum* and *F. meridionale*. The number of isolates was not only increased, but isolates were collected from both maize and wheat and they were evaluated individually rather than in mixtures. Our goal was to determine whether this larger survey would confirm our previous results suggesting that *F. meridionale* was more aggressive on maize ears than *F. graminearum*.

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 these experiments. The isolates were previously identified simultaneously to species and trichothecene chemotypes (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 isolates were selected to be representative geographically and for time 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 1.

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).

Table 1. Summary information for 41 isolates used in this study, 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.

Species	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 tricothecene 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.

Field experiments

Field experiments were conducted at the experimental station at the Universidade Federal de Viçosa (20°44'44" S, 42°50'59" 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) maize hybrids were planted on 4th and 25th of April, and harvested on 18th of August and 25th of September, respectively. For the 2018 growing season, the maize hybrids MG580PW[®] (Dow AgroSciences) and BM820[®] (Sementes Biomatrix) were planted on 25th of October and 20th of November 2017, and harvested on 11th of February and 15th of March 2018, respectively. Two trials were planted side-by-side, one for the GER assay and the other for the GSR assay. The entire field was fertilized following chemical soil analyses, and sprinkle-irrigated as needed.

The experiment was arranged as a randomized complete block design with four replications. The isolates were randomly assigned to each experimental unit, which consisted of a 1-m-long row. The central three plants in each row were

inoculated. All units were bordered by non-inoculated 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 a 5 cm long and 3 mm diameter obtuse needle (Reid et al. 2002). Ears mock-inoculated with sterile distilled water served as negative controls. The 2017 trials were inoculated on 15th of June and 7th of August, while the 2018 trials were inoculated on 5th of January and 9th of February.

The plots were irrigated for two consecutive days after inoculation. When plants reached the R5-R6 stage (dent to physiological maturity), ears were hand-picked and husked. GER severity was scored visually as the percent symptomatic area of each ear (Reid and Zhu 2005).

GSR trials. Maize stalks were also inoculated four days after silk emergence. One milliliter 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). 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. The percent diseased (discolored) area of the internode (Reid and Zhu 2005) was measured by using Assess software (Lamari 2002).

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 ($\log\text{SEV} = \log[\text{SEV} + 1]$) 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 intraspecies variation in symptom severity, and ranges overlapped between the two species (Fig. 1). Mean severity differed between the species, but this 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* ($P = 0.25$). 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 7.2% (± 5.3 standard error [SE]) and 13.9% (± 9.4 SE), respectively (Fig. 3A). 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 10.6% (± 7.3 SE) and 11.8% (± 8.1 SE) for *F. graminearum* and *F. meridionale*, respectively (Fig. 3B).

Figure 1. 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 represent the mean GER severity for each isolate across all inoculated ears. 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 the 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.

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 81.2% (median = 18.5%) (Fig. 2). 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 ($18.4\% \pm 7.1$ SE) than *F. meridionale* ($16.1\% \pm 6.2$ SE) (Fig. 3B). There was no significant correlation between stalk and ear disease severity ($\rho = -0.10$; $P = 0.218$).

Figure 2. 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 represent the mean GSR severity for each isolate across all inoculated stalks. 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 the 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.

Figure 3. 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 the 2017 growing season and the last two were cultivated during the 2018 growing season.

Mycotoxin analysis

Mycotoxins were analyzed in maize hybrid BM820® kernels harvested from the experiment conducted in 2018. 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. 4A).

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. 4). None of the other *F. graminearum* strains produced detectable 3ADON. On average, *F. graminearum* strains from wheat produced more than 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).

There was a significant correlation between GER severity and DON production by *F. graminearum* isolates ($\rho = 0.81$; $P = 0.027$). For *F. meridionale* isolates,

a significant positive correlation was also observed between GER severity and NIV production ($\rho = 0.79$; $P < 0.01$) (Fig. 4).

Figure 4. (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 represent a bulked sample of kernels from 5-6 inoculated ears. Limit of detection ($< 0.25 \mu\text{g/g}$).

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 was 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; Valverde-Bogantes et al., 2019) have also been implicated as important drivers of rapid changes in relative dominance of species among pathogenic populations. The importance of the host on FGSC composition has emerged more recently from work done 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 shown that *F. meridionale* is dominant as a maize pathogen, while *F. graminearum* is dominant as a wheat pathogen in regions where these species and crops co-occur. Results of the study reported here support the hypothesis that increased aggressiveness of *F. meridionale* to maize ears plays a role in host dominance, given that *F. meridionale* strains caused twice as much GER, on average, as *F. graminearum*. Closer scrutiny of the data revealed that this difference occurred only when those strains originated from maize, and not when they came from wheat. This result was consistent with our previous work in which a more limited sample of *F. meridionale* strains only from maize were also twice as aggressive as the *F. graminearum* strains that also came from maize (Machado et al., 2021). This host effect could be due to differential selection, either selection for more aggressive *F. meridionale* strains by maize, or for more aggressive *F. graminearum* strains by wheat, or a combination of the two.

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). Alarming, NIV is about 10 times more toxic to animals than DON (Desjardins 2008). The results presented herein confirm our previous findings, demonstrating that *F. meridionale* isolates causing GER produce NIV in maize ears (Machado et al., 2021). 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; 2017). *Fusarium graminearum*-inoculated ears contained DON and no NIV, but levels of DON were seven times higher than NIV levels. In this study, evaluation of more isolates, and inoculating them separately instead of pooling them, showed that both NIV and DON were positively 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 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 may be more important for wheat colonization. A role for DON in colonization of maize is known (Bai et al. 2002; Desjardins et al. 1996; Harris et al. 1999; Maier et al. 2006). However, the role of NIV mycotoxin in pathogenesis on maize is less understood and needs to be further explored. Not all of the strains tested produced detectable levels of mycotoxins in the composite samples of kernels from field inoculations. Low levels of mycotoxin were

likely undetected in those cases due to technical limitations of the sampling and detection methods. The ability of the genotyping to predict the chemotype was confirmed here (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 when stalks were inoculated with either species under controlled conditions. Intriguingly, it was 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 reported here may be related to the influence of environmental factors or host age, both of which could increase susceptibility to *F. graminearum* relative to *F. meridionale*. There may be other reasons for the dominance of *F. meridionale* on maize stalks in the field, including inoculum availability and differential responses to environmental factors during perithecial

formation or overwintering survival. *F. meridionale* has been demonstrated to be more competitive in maize ears during mixed infections (Machado et al., 2021), and so another explanation for dominance of *F. meridionale* perithecia on maize stalks could be that it outcompetes *F. graminearum* during stalk colonization when both species are present.

Resistance to GSR and GER in maize is complex, and is influenced by genetic background as well as 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). We found no correlation in the degree of aggressiveness of individual isolates to ears versus stalks. A previous study 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.

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REFERENCES

- Anderson, N. R., Ravellette, J. D., and Wise, K. A. 2016. Improved method for injecting fungal inoculum into corn ears. *Plant Health Prog.* 17:163–166.
- Andriolli, C. F., Casa, R. T., Kuhnem, P. R., Bogo, A., Zancan, R. L., and Reis, E. M. 2016. Timing of fungicide application for the control of *Gibberella* ear rot of maize. *Trop Plant Pathol.* 41:264–269.
- Astolfi, P., dos Santos, J., Schneider, L., Gomes, L. B., Silva, C. N., Tessmann, D. J., et al. 2011. Molecular survey of trichothecene genotypes of *Fusarium graminearum* species complex from barley in Southern Brazil. *Int. J. Food Microbiol.* 148:197–201.
- Bai, G.-H., Desjardins, A., and Plattner, R. 2002. Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. *Mycopathologia.* 153:91–98.
- Bates, D., Mächler, M., Bolker, B., and Walker, S. 2015. Fitting linear mixed-effects

- models using lme4. J. Stat. Softw. 67 Available at: <http://www.jstatsoft.org/v67/i01/> [Accessed October 4, 2019].
- Boutigny, A.-L., Ward, T. J., Van Coller, G. J., Flett, B., Lamprecht, S. C., O'Donnell, K., et al. 2011. Analysis of the *Fusarium graminearum* species complex from wheat, barley and maize in South Africa provides evidence of species-specific differences in host preference. Fungal Genet. Biol. 48:914–920.
- Carter, J. P., Rezanoor, H. N., Desjardins, A. E., and Nicholson, P. 2000. Variation in *Fusarium graminearum* isolates from Nepal associated with their host of origin. Plant Pathol. 49:452–460.
- Castañares, E., Dinolfo, M. I., Del Ponte, E. M., Pan, D., and Stenglein, S. A. 2016. Species composition and genetic structure of *Fusarium graminearum* species complex populations affecting the main barley growing regions of South America. Plant Pathol. 65:930–939.
- Chungu, C., Mather, D., Reid, L., and Hamilton, R. 1996. Comparison of techniques for inoculating maize silk, kernel, and cob tissues with *Fusarium graminearum*. Plant Dis. 80:81–84.
- Costa, R. V. da, Simon, J., Cota, L. V., Silva, D. D. da, Almeida, R. E. M. de, Lanza, F. E., et al. 2019. Yield losses in off-season corn crop due to stalk rot disease. Pesqui. Agropecuária Bras. 54.
- Cumagun, C. J. R., and Miedaner, T. 2004. Segregation for aggressiveness and deoxynivalenol production of a population of *Gibberella zeae* causing head blight of wheat. Eur. J. Plant Pathol. 110:789–799.
- Del Ponte, E. M., Fernandes, J. M. C., Pavan, W., and Baethgen, W. E. 2009. A Model-based assessment of the impacts of climate variability on Fusarium Head Blight seasonal risk in southern Brazil. J. Phytopathol. 157:675–681.
- Del Ponte, E. M., Garda-Buffon, J., and Badiale-Furlong, E. 2012. Deoxynivalenol and nivalenol in commercial wheat grain related to Fusarium head blight epidemics in southern Brazil. Food Chem. 132:1087–1091.

- Del Ponte, E. M., Spolti, P., Ward, T. J., Gomes, L. B., Nicolli, C. P., Kuhnem, P. R., et al. 2015. Regional and field-specific factors affect the composition of *Fusarium* Head Blight pathogens in subtropical no-till wheat agroecosystem of Brazil. *Phytopathology*. 105:246–254.
- Desjardins, A. E. 2008. Natural product chemistry meets genetics: when is a genotype a chemotype? *J. Agric. Food Chem.* 56:7587–7592.
- Desjardins, A. E., Proctor, R. H., Bai, G., McCormick, S. P., Shaner, G., Buechley, G., et al. 1996. Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. *Mol. Plant. Microbe Interact.* 9:775–781.
- Fuentes, R. G., Mickelson, H. R., Busch, R. H., Dill-Macky, R., Evans, C. K., Thompson, W. G., et al. 2005. Resource allocation and cultivar stability in breeding for *Fusarium* Head Blight resistance in spring wheat. *Crop Sci.* 45:1965–1972.
- Gai, X. T., Xuan, Y. H., and Gao, Z. G. 2017. Diversity and pathogenicity of *Fusarium graminearum* species complex from maize stalk and ear rot strains in northeast China. *Plant Pathol.* 66:1267–1275.
- Gomes, L. B., Ward, T. J., Badiale-Furlong, E., and Del Ponte, E. M. 2015. Species composition, toxigenic potential and pathogenicity of *Fusarium graminearum* species complex isolates from southern Brazilian rice. *Plant Pathol.* 64:980–987.
- Harris, L. J., Desjardins, A. E., Plattner, R. D., Nicholson, P., Butler, G., Young, J. C., et al. 1999. Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. *Plant Dis.* 83:954–960.
- Hothorn, T., Bretz, F., and Westfall, P. 2008. Simultaneous inference in general parametric models. *Biom. J.* 50:346–363.
- Kazan, K., Gardiner, D. M., and Manners, J. M. 2012. On the trail of a cereal killer: recent advances in *Fusarium graminearum* pathogenomics and host resistance. *Mol. Plant Pathol.* 13:399–413.
- Kelly, L. A., Tan, Y. P., Ryley, M. J., and Aitken, E. A. B. 2017. *Fusarium* species

- associated with stalk rot and head blight of grain sorghum in Queensland and New South Wales, Australia. *Plant Pathol.* 66:1413–1423.
- Kuhnem Júnior, P. R., Stumpf, R., Spolti, P., and Ponte, E. M. D. 2013. Características patogênicas de isolados do complexo *Fusarium graminearum* e de *Fusarium verticillioides* em sementes e plântulas de milho. *Ciênc. Rural.* 43:583–588.
- Kuhnem, P. R., Del Ponte, E. M., Dong, Y., and Bergstrom, G. C. 2015. *Fusarium graminearum* isolates from wheat and maize in New York show similar range of aggressiveness and toxigenicity in cross-species pathogenicity tests. *Phytopathology.* 105:441–448.
- Kuhnem, P. R., Ward, T. J., Silva, C. N., Spolti, P., Ciliato, M. L., Tessmann, D. J., et al. 2016. Composition and toxigenic potential of the *Fusarium graminearum* species complex from maize ears, stalks and stubble in Brazil. *Plant Pathol.* 65:1185–1191.
- Lamari, L. 2002. Assess: Image Analysis Software for Plant Disease Quantification. American Phytopathological Society Press, St. Paul, Mn.
- Lenth, R. 2019. *emmeans: Estimated Marginal Means, aka Least-Squares Means*. Available at: <https://CRAN.R-project.org/package=emmeans>.
- Liu, Y., Sun, H., Li, W., Xia, Y., Deng, Y., Zhang, A., et al. 2017. Fitness of three chemotypes of *Fusarium graminearum* species complex in major winter wheat-producing areas of China ed. Yin-Won Lee. *PLOS ONE.* 12:e0174040.
- Logrieco, A., Mulè, G., Moretti, A., and Bottalico, A. 2002. Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe eds. A. Logrieco, J. A. Bailey, L. Corazza, and B. M. Cooke. *Eur. J. Plant Pathol.* 108:597–609.
- Maier, F. J., Miedaner, T., Hadel, B., Felk, A., Salomon, S., Lemmens, M., et al. 2006. Involvement of trichothecenes in fusarioses of wheat, barley and maize evaluated by gene disruption of the trichodiene synthase (Tri5) gene in three field isolates of different chemotype and virulence. *Mol. Plant Pathol.* 7:449–461.

- McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., et al. 2012. A unified effort to fight an enemy of wheat and barley: Fusarium Head Blight. *Plant Dis.* 96:1712–1728.
- Mesterházy, Á., Lemmens, M., and Reid, L. M. 2012. Breeding for resistance to ear rots caused by *Fusarium* spp. in maize-a review. *Plant Breed.* 131:1–19.
- Miller, J. D., and Greenhalgh, R. 1991. Trichothecene chemotypes of three *Fusarium* species. *Mycologia.* 83:121–130.
- Mirocha, C. J., Kolaczowski, E., Xie, W., Yu, H., and Jelen, H. 1998. Analysis of deoxynivalenol and Its derivatives (batch and single kernel) using gas chromatography/mass spectrometry. *J. Agric. Food Chem.* 46:1414–1418.
- Nicholson, P., Simpson, D. R., Weston, G., Rezanoor, H. N., Lees, A. K., Parry, D. W., et al. 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. *Physiol. Mol. Plant Pathol.* 53:17–37.
- Nicolli, C. P., Machado, F. J., Spolti, P., and Del Ponte, E. M. 2018. Fitness traits of deoxynivalenol and nivalenol-producing *Fusarium graminearum* species complex strains from wheat. *Plant Dis.* 102:1341–1347.
- Nicolli, C. P., Spolti, P., Tibola, C. S., Fernandes, J. M. C., and Del Ponte, E. M. 2015. Fusarium head blight and trichothecene production in wheat by *Fusarium graminearum* and *F. meridionale* applied alone or in mixture at post-flowering. *Trop. Plant Pathol.* 40:134–140.
- Oldenburg, E., Höppner, F., Ellner, F., and Weinert, J. 2017. *Fusarium* diseases of maize associated with mycotoxin contamination of agricultural products intended to be used for food and feed. *Mycotoxin Res.* 33:167–182.
- Oliveira, M. S., Rocha, A., Sulyok, M., Krska, R., and Mallmann, C. A. 2017. Natural mycotoxin contamination of maize (*Zea mays* L.) in the South region of Brazil. *Food Control.* 73:127–132.
- Picot, A., Hourcade-Marcolla, D., Barreau, C., Pinson-Gadais, L., Caron, D., Richard-Forget, F., et al. 2012. Interactions between *Fusarium verticillioides* and

- Fusarium graminearum* in maize ears and consequences for fungal development and mycotoxin accumulation. *Plant Pathol.* 61:140–151.
- Quesada-Ocampo, L. M., Al-Haddad, J., Scruggs, A. C., Buell, C. R., and Trail, F. 2016. Susceptibility of maize to stalk rot caused by *Fusarium graminearum* deoxynivalenol and zearalenone mutants. *Phytopathology*. 106:920–927.
- R Core Team. 2019. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at: <http://www.R-project.org/>.
- Reid, L. M., Woldemariam, T., Zhu, X., Stewart, D. W., and Schaafsma, A. W. 2002. Effect of inoculation time and point of entry on disease severity in *Fusarium graminearum*, *Fusarium verticillioides*, or *Fusarium subglutinans* inoculated maize ears. *Can. J. Plant Pathol.* 24:162–167.
- Reid, L. M. and Zhu, X. 2005. Screening corn for resistance to common diseases in Canada. Agriculture and Agri-Food Canada, Ottawa, 29p.
- Umpierrez-Failache, M., Garmendia, G., Pereyra, S., Rodríguez-Haralambides, A., Ward, T. J., and Vero, S. 2013. Regional differences in species composition and toxigenic potential among *Fusarium* head blight isolates from Uruguay indicate a risk of nivalenol contamination in new wheat production areas. *Int. J. Food Microbiol.* 166:135–140.
- Vaughan, M., Backhouse, D., and Ponte, E. M. D. 2016. Climate change impacts on the ecology of *Fusarium graminearum* species complex and susceptibility of wheat to *Fusarium* head blight: a review. *World Mycotoxin J.* 9:685–700.
- Ward, T. J., Clear, R. M., Rooney, A. P., O'Donnell, K., Gaba, D., Patrick, S., et al. 2008. An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. *Fungal Genet. Biol.* 45:473–484.
- Yang, Q., Yin, G., Guo, Y., Zhang, D., Chen, S., and Xu, M. 2010. A major QTL for resistance to *Gibberella* stalk rot in maize. *Theor Appl Genet.* 121:673–687.

SUPPLEMENTARY MATERIAL

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 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 the 2018 growing season, respectively.