

Chapter 2

Species Identification, Genetic Diversity and Phenotypic Variation Studies on the *Fusarium graminearum* Complex Populations from Brazil

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Abstract *Fusarium graminearum* sensu lato, the cause of Fusarium head blight (FHB), has been reported for more than 50 years in Brazil. However, it was only during the mid-1980s to late 1990s that FHB resurged as a primary concern of wheat production. More than a dozen studies in Brazil or elsewhere have been conducted since the year 2000 employing novel and accurate methods to explore genetic variability and identify the strains to phylogenetic species. Thus far, six species of the *F. graminearum* species complex (FGSC) have been reported in association with wheat, barley, oat, soybean, maize, rice and ryegrass in Brazil, and their prevalence rates appears to be dependent on the region and host surveyed. Although *F. graminearum* sensu stricto (s. str.) is dominant in wheat, *F. meridionale* is the most frequently found pathogen in maize, and *F. asiaticum* is the only species found in rice. Most of them preferentially exhibit specific trichothecene genotypes: *F. graminearum* s. str. is of the 15-acetyl-deoxynivalenol (DON) genotype, *F. meridionale* and *F. asiaticum* are of the nivalenol (NIV) genotype, and *F. austroamericanum* is of the 3-ADON genotype; an exception is that *F. cortaderiae* exhibits either the 3-ADON or NIV genotype. They have also exhibited differences in relation to their reproductive fitness, fungicide sensitivity and pathogenicity toward wheat and maize. Continuous monitoring of the genotypic and phenotypic traits of the FGSC populations will help to improve local disease management and contribute to the global knowledge of the biology of the pathogen.

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2.1 Introduction

Fusarium graminearum sensu lato (teleomorph *Gibberella zeae*) is the main cause of Fusarium head blight (FHB), a devastating and resurgent disease of wheat both in Brazil and worldwide (Goswami and Kistler 2004). The fungus also causes stalk and ear rot of maize (Sutton 1982) and may survive in the seeds or residues of several other cereal and non-cereal crops grown in succession or rotation with wheat and barley, such as rice, soybean and several grasses (Parry et al. 1995). In Brazil, two factors are key determinants of FHB risk: intensive no-till systems that contribute to the maintenance of high levels of inoculum and a wet subtropical climate that usually favors FHB in most years (Del Ponte et al. 2009). The fungus is known to produce relatively large amounts of deoxynivalenol (DON) and, ultimately, nivalenol (NIV), two trichothecene mycotoxins that may accumulate in grains at levels considered unsafe for both human and animal consumption (Creppy 2002).

In spite of the resurgence of FHB as a major threat to wheat production in Brazil in the early 1990s, it was only in 2011 that maximum tolerance limits were promulgated for *Fusarium* trichothecene mycotoxins, more specifically for DON, in the grains and by-products of wheat, barley, rice and other crops grown in the country. However, published data for the mycotoxin contamination of commercial wheat grains in Brazil is scarce, and the most recent reports have shown the co-occurrence of DON and NIV mycotoxins in commercial grains at levels that exceed the 2 ppm threshold. Furthermore, it was found that the trichothecene content in grains can vary from 1 year to the next and also according to the region surveyed, demonstrating that the local populations are able to produce both trichothecenes (Del Ponte et al. 2012).

Based on phylogenetic species recognition, with genealogical concordance (Taylor et al. 2000), taxonomic studies in the last decade have provided evidence that *F. graminearum* sensu lato comprises at least 15 phylogenetically distinct species, also known as the *F. graminearum* species complex (FGSC) (O'Donnell et al. 2004; Sarver et al. 2011). Additionally, chemical and/or molecular analyses have been performed in some of these studies to determine the trichothecene chemotype and/or genotype composition of FGSC (Ward et al. 2002; de Kuppler et al. 2011; Reynoso et al. 2011). In addition, the genetic diversity and population structure across various spatial and temporal scales have been assessed in the FGSC populations worldwide using a variety of molecular markers, as recently reviewed (Wang et al. 2011). Phenotypic traits, such as reproductive fitness, fungicide sensitivity and pathogenicity, have also been assessed comparatively among FGSC members (Goswami and Kistler 2005; Lamprecht et al. 2011; Spolti et al. 2012a; Shen et al. 2012).

Studies in both North and South America have shown that *F. graminearum* sensu stricto (s. str), formerly known as lineage 7, mainly a DON producer, appears to be the dominant species associated with the head blight of wheat and barley (Ward et al. 2008; Alvarez et al. 2011; Schmale et al. 2011; Astolfi et al. 2012a). Other species of the complex are either limited geographically or appear to exhibit a possible host preference (O'Donnell et al. 2004; Sarver et al. 2011; Astolfi et al. 2012b).

In this chapter, we review the literature and summarize the data from studies conducted in Brazil, or elsewhere with strains from Brazil, to determine the species

diversity, genetic diversity and phenotypic traits of the FGSC populations. Our focus is not limited to the strains causing head blight of wheat and barley but also include those infecting or surviving on the seeds and residues of other economically important crops, such as maize, soybean, and rice, which may serve as inoculum sources for epidemics in winter cereal crops. It is expected that such information can provide valuable insight into the ecological and evolutionary aspects of the FGSC populations in Brazil and contribute to the global knowledge of the pathogen.

2.2 Species Identification Based on Morphological and Reproductive Traits

In Brazil, the first FHB studies date from the 1960s in Rio Grande do Sul (RS) State where the disease caused by *F. graminearum* was first recorded, reportedly during the early 1940s (Costa Neto 1947). The plant pathological herbarium of the Universidade Federal do Rio Grande do Sul (UFRGS) contains more than 50 records of specimens of *F. graminearum* associated with wheat FHB, which were collected and deposited by Prof. Costa Neto, a plant pathologist and professor at UFRGS from 1941 to the early 1970s. The initial research began in 1960 at the Faculdade de Agronomia Eliseu Maciel and the Instituto de Pesquisas e Experimentação Agropecuária do Sul (further incorporated by Embrapa) by means of a Brazil-Japan collaboration that included exchange visits and the introduction of resistant varieties and breeding with local materials (Silva 1966; Luzzardi et al. 1972, 1974).

When Embrapa Trigo, the national wheat research center was founded in Passo Fundo, RS during the early 1970s, further studies were conducted with regard to the epidemiology and management of FHB (Reis 1986a b, 1990; Lima et al. 1998, 2000; Picinini and Fernandes 1998). Although there are records of *F. avenaceum* (Reis 1988) and *F. tricinctum* (Pierobom et al. 1977) associated with head blight symptoms, *F. graminearum* appears to be the principal causal agent of the head blight of wheat and barley in Brazil. During surveys conducted in the 1980s, *F. graminearum* Group 1, now recognized as a separate species, *F. pseudograminearum* (Aoki and O'Donnel 1999), had not been found (Reis 1986a, 1990), and no further study has confirmed its occurrence in Brazil to date.

During the mid to late 1990s, a time when FHB was no longer considered a secondary disease of wheat in Brazil, as it was in the two previous decades, researchers became more interested in exploring the variability of the putative *F. graminearum* populations causing head blight of wheat and barley or associated with other hosts. By 2012, a variety of methods had been used in 16 published studies, including scientific articles and theses, which were aimed at identifying species in the complex or to within the complex level, in addition to the genetic diversity and population structure (Fig. 2.1).

The earliest and most comprehensive study for the identification of *Fusarium* spp. associated with FHB and other hosts was based on morphology and reproduction and included 85 isolates from Embrapa Trigo collected in Rio Grande do Sul

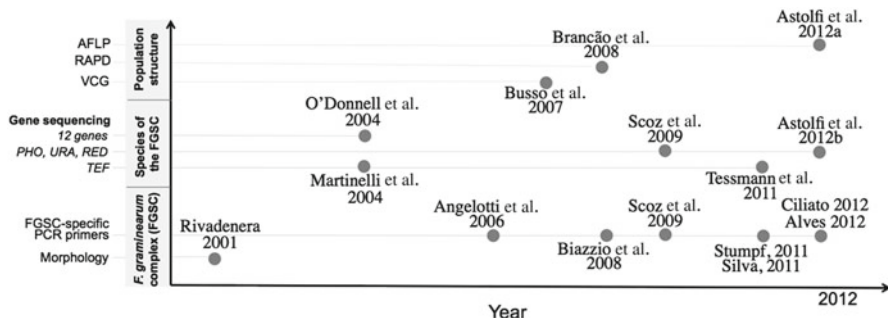


Fig. 2.1 Timeline of 16 studies conducted within a decade (2001–2012) by year of publication and the different methods used for the species identification and genetic diversity analysis of *Fusarium graminearum* species complex strains associated with agricultural crops in Brazil

State during the mid- to late 1990s. Based on morphological (reverse and surface colony color, abundance of mycelia and presence of macro- and microconidia), reproductive (mycelial growth rate and perithecia production) and pathogenic traits, all but ten isolates were identified as *F. graminearum* (Rivadenera 2001).

2.3 Molecular Identification and Genetic Diversity

The first Brazilian study using PCR (polymerase chain reaction) for the identification of *Fusarium* spp. associated with FHB was published in the mid-2000s using a limited number of isolates ($n=16$) obtained from symptomatic wheat kernels and four isolates from triticale crops grown in southern Brazil (Angelotti et al. 2006). All the isolates amplified the expected fragment size for the UBC85 primer that targets the *F. graminearum* complex. In a follow-up study from the same group, Biazio et al. (2008) identified a sub-set of 14 *F. graminearum* isolates, previously identified with the UBC85 F/R primer, using a new PCR assay developed by the authors for the identification of *F. graminearum*. The new pair of primers, named GO F/R, targeted a segment of the 3' coding region of the *gaoA* gene that codes for the enzyme galactose oxidase (GO) and proved to be specific to *F. graminearum* complex strains. The GO primer pair, in addition to the UBC85 and Fg16 primers, were used in PCR assays for detecting *F. graminearum* in spiked or naturally infected grains of bulgur wheat, but only one *F. graminearum* strain was found in commercial grain samples (Faria et al. 2012).

In Southern Paraná (PR) State, a small collection of nine monosporic isolates obtained from symptomatic wheat heads of six cultivars grown at an experimental station during the 2004 epidemic year was studied using VCG (vegetative compatibility groups based on nitrate non-utilizing mutants) and RAPD analysis. Although VCG separated the isolates into three groups, the UPGMA analysis using profiles of eight RAPD primers separated the isolates into two groups containing seven and

two isolates each, leading the authors to conclude that the population in their study was fairly clonal (Busso et al. 2007).

In RS State, 20 strains obtained from fields at a different municipalities of the northern region of the state during the 2005 growing season were identified as *F. graminearum* using PCR-based assays with TOX5 and GaoA-V2 primers that target potential trichothecene-producing strains of *Fusarium* and galactose oxidase-producing *F. graminearum*, respectively (Brancão et al. 2008). Additionally, the analysis based on 102 polymorphic RAPD markers separated the isolates into two distinct groups, which were, on average, 52 % similar. The first and larger group, which included 17 isolates, showed >80 % similarity, and the second group of three strains showed 60–75 % similarity among them (Brancão et al. 2008).

All the above studies conducted by Brazilian researchers have confirmed the identification of isolates at the *F. graminearum* species complex level, though not at the lineage or phylogenetic species levels, as defined by O'Donnell et al. (2004). However, there are a few recent reports of the occurrence of those lineages or species of the *F. graminearum* complex in Brazil being associated with several hosts. O'Donnell et al. (2004) first recognized nine phylogenetic species among the named *F. graminearum* isolates obtained from oat seeds in southern Brazil as *F. brasiliicum*. According to their sampling of isolates from Brazil, other species would be endemic to the country, such as *F. graminearum* s. str., *F. austroamericanum*, *F. meridionale*, *F. cortaderiae* and *F. asiaticum* (O'Donnell et al. 2004).

Martinelli et al. (2004) identified to the lineage level a small collection of six isolates obtained from soybean seeds grown in PR and RS States, and one isolate obtained from wheat grown in PR State by amplifying a portion of the gene encoding translation elongation factor (TEF) and comparing the DNA sequences to those previously published from strains of known lineages. Although the only isolate from wheat was identified as lineage 8 (*F. cortaderiae*), one isolate from soybean was classified as lineage 1 (*F. austroamericanum*), three as lineage 2 (*F. meridionale*) and two as lineage 8 (*F. cortaderiae*) (Martinelli et al. 2004).

The first comprehensive molecular survey of isolates obtained at 20 locations in the major wheat-producing regions in southern Brazil was conducted during the 2006 growing season and resulted in the analysis of 82 *F. graminearum* monosporic isolates obtained from damaged wheat kernels (Scoz et al. 2009). The data from PCR assays using the Fg16 F/R, a primer pair that produces a polymorphic band for *F. graminearum* complex isolates, showed the presence of two SCAR groups: a larger group of 76 isolates produced a PCR amplicon of 450 bp, and six isolates generated an amplicon of 490 bp. To assist in the identification to the phylogenetic species level of representative isolates of these two groups, portions of the PHO, RED and URA genes from seven isolates of these groups were analyzed. Blast queries for individual gene sequences and pairwise comparisons of percentage identity based on 1676 bp of concatenated DNA sequence showed that the three representatives of the larger group were 98.7 % similar to reference isolates of *F. graminearum* s. str. and that the other four isolates were 98.43 % similar to reference isolates of *F. meridionale*. In a follow-up study, 140 isolates obtained in northern RS State during the 2007 growing season were split into three populations, each from wheat

fields at least 150 km from the others, and examined using the Fg16F/R primer pair and AFLP markers (Astolfi et al. 2012a). Based on the PCR SCAR groups, *F. graminearum* s. str. was the dominant population, followed by *F. meridionale*, with frequency levels ranging from 2 to 18 % across the fields. The genetic diversity assessed in a sample of 103 *F. graminearum* s. str. isolates using amplified fragment length polymorphism (AFLP) markers showed that the isolates with the same haplotype were rare and that the genotypic diversity was uniformly high (≥ 98 %), suggesting that sexual recombination has played a significant role in this group. The number of migrants was estimated between 5 and 6 across all loci and all populations, but the high frequency of private alleles (up to 30 %) suggested a historical, rather than a contemporary, gene flow. Regarding linkage disequilibrium, 0.8, 1.5 and 2.2 % of the locus pairs from the three populations were in disequilibrium, which is lower than the values reported in other regions of the world (Astolfi et al. 2012a).

The first survey of *F. graminearum* strains associated with barley kernels produced in Brazil was conducted during 3 years (2007, 2008 and 2009) using samples obtained from several fields in both southern and northern production regions of Rio Grande do Sul State, Brazil. Evidence based on the PCR SCAR groups for 92 isolates and portions of the PHO, RED and URA genes for a subsample of 8 isolates of the different groups and trichothecene genotypes showed the occurrence of *F. graminearum* s. str. as the dominant species, followed by *F. meridionale*, with a frequency higher than that found for the strains from wheat, and *F. austroamericanum* at a very low frequency (Astolfi et al. 2012b).

In maize, *F. graminearum* sensu lato has more commonly been found in South Brazil, a subtropical region, and less commonly in the tropical highlands in the Central Brazil, a region where *F. verticilloides* is more prevalent (Silva 2011; Tessmann et al. 2011). The identification of 105 monosporic isolates obtained from maize kernels from fields in both Central and South Brazil based on the partial sequence of the TEF gene revealed different species of FGSC: *F. meridionale*, the dominant species (73 %), *F. graminearum* s. str. (14 %), and *F. cortaderiae* (13 %), which is limited geographically to the southernmost part of the country (Tessmann et al. personal communication).

Thus far, six species of the *F. graminearum* species complex (FGSC) have been reported in association with important agricultural crops such as wheat, barley, oat, soybean, maize, rice and ryegrass in Brazil, and the predominance of these species appears to be dependent on the region and host surveyed (Table 2.1).

2.4 Trichothecene-Producing Potential

The FGSC populations from Brazil have also been studied with regard to their mycotoxin potential. The first study on mycotoxin occurrence in wheat grains produced in Brazil has provided indirect evidence that *F. graminearum* populations, which are the main trichothecene-producing fungi infecting wheat, were capable of producing DON and NIV, as based on the detection of these mycotoxins in wheat

Table 2.1 Phylogenetic species (former lineage) and the respective trichothecene genotype determined by molecular analyses or the chemotype* determined by chemical analysis of isolates of the *Fusarium graminearum* species complex associated with agricultural crops grown in Brazil

FGSC species	Host	Trichothecene	Reference
<i>F. austroamericanum</i> (Lineage 1)	Soybean	DON*	Martinelli et al. (2004)
	Maize kernel	3-ADON*	O'Donnell et al. (2004)
	Barley	3-ADON	Astolfi et al. (2012b)
<i>F. meridionale</i> (Lineage 2)	Soybean	NIV*	Martinelli et al. (2004)
	Wheat	NIV	Scoz et al. (2009)
	Maize kernel	NIV	Silva (2011)
	Maize kernel	NIV	Stumpf (2011)
	Maize stubble	NIV	Kuhnem et al.*
<i>F. asiaticum</i> (Lineage 6)	Oat	NIV	O'Donnell et al.*
	Rice	NIV	Gomes et al.*
	Ryegrass	NIV	Del Ponte et al.*
	Wheat	NIV	Del Ponte et al.*
<i>F. graminearum</i> (Lineage 7)	Wheat, oat, barley, soybean	15-ADON	O'Donnell et al.*
	Wheat	15-ADON	Scoz et al. (2009)
	Wheat	15-ADON	Astolfi et al. (2012a)
	Barley	15-ADON	Astolfi et al. (2012b)
	Maize	15-ADON	Silva et al.*
	Maize stubble	15-ADON	Kuhnem et al.*
	Ryegrass	15-ADON	Del Ponte et al.*
<i>F. cortaderiae</i> (Lineage 8)	Barley, wheat	3-ADON	O'Donnell et al.*
	Wheat, soybean	NIV*	Martinelli et al. (2004)
	Maize kernel	NIV, 3-ADON	Silva (2011)
	Maize stubble	NIV, 3-ADON	Kuhnem et al.*
	Ryegrass	NIV	Del Ponte et al.*
<i>F. brasilicum</i>	Oat	NIV*	O'Donnell et al. (2004)

*Personal communication

grains (Furlong et al. 1995). This result has been confirmed recently with the detection of both DON and NIV at similar levels in commercial wheat grain from RS State (Del Ponte et al. 2012).

Strains of FGSC can be classified as possessing a specific trichothecene chemotype (chemical phenotype) according to the chemical analysis of artificial or natural substrates, a method based on which trichothecene is predominantly produced and its abundance (Desjardins et al. 1993). Trichothecene genotypes have been identified with the aid of PCR assays employing a suite of primers directed to portions of genes (*TRI3*, *TRI5*, *TRI7*, *TRI12*, and *TRI13*) that discriminate the genotypes of NIV, DON and its two acetylates: 3-ADON and 15-ADON (Chandler et al. 2003; Ward et al. 2002; Quarta et al. 2006).

Chemotypes and/or genotypes of the FGSC strains from Brazil have been determined concomitantly with species identification or for a set of known species. In a multi-toxin production study, thin-layer chromatography was used to detect and identify trichothecene (DON, NIV, diacetoxyscirpenol, fusarenon-X, neosolaniol

and NIV), zearalenone and zearalenol production on a rice substrate by 24 FGSC isolates that were obtained from wheat (n=19), barley (n=1) and triticale (n=4) grown in southern Brazil. The authors observed that 67 % of the isolates produced zearalenone and 33 % produced DON. In addition, a few isolates also produced zearalenol, diacetoxyscirpenol and fusarenon-X, though no isolate produced nivalenol or neosolaniol (Geraldo et al. 2006).

For five strains from soybean and one from wheat identified as species of FGSC, a GC/MS (gas chromatography/mass spectrometry) analysis showed that all the isolates were capable of producing DON and/or NIV, which were detected 2 weeks after spraying the anthers of the wheat heads with a spore suspension. Strains of lineages 2 (*F. meridionale*) and 8 (*F. cortaderiae*) were essentially NIV producers but also produced small amounts of DON, whereas the lineage 1 strain (*F. austroamericanum*) produced only DON in very large amounts. It was also observed that the nivalenol chemotypes were also capable of producing 3-acetyl nivalenol (Martinelli et al. 2004).

Trichothecene genotypes have been determined mostly based on the amplification of portions of the *TRI3* and *TRI2* genes in a multiplex PCR assay and some using the *TRI3* gene that differentiates the DON and NIV genotypes. The first report of trichothecene genotypes for a large number of strains (n=82) surveyed across wheat regions in South Brazil was conducted by Scoz et al. (2009), and the authors identified six NIV and 76 15-ADON genotypes based on primers targeting the *TRI3*, *TRI2* and *TRI3* genes. In an ensuing study with higher number of strains from the same field, Astolfi et al. (2012a) also identified the 15-ADON and NIV genotypes in wheat field populations surveyed in 2007 at three locations in Northern RS State. A large and variable predominance of 15-ADON across the locations was found. A fine-scale survey of the isolates obtained from several georeferenced locations within a field was conducted in 2008, and three genotypes were found, with a tendency for the less frequent genotypes (NIV and 3-ADON) to aggregate spatially (Astolfi et al. 2010). Three genotypes were also found for the FGSC strains from barley, but, differently from wheat, the NIV genotype was found at 3× higher frequency in barley, along with the detection of a few 3-ADON isolates (Astolfi et al. 2012b). The trichothecene genotype diversity among strains examined has been associated with species differences: the *F. graminearum* s. str. strains were all of the 15-ADON genotype; the *F. meridionale* strains were of the NIV genotype; and the *F. austroamericanum* strains were of the 3-ADON genotype (Table 2.1). In Brazil, the FGSC populations from maize have also been under investigation and, as in wheat, the species were also correlated to specific genotypes: *F. meridionale* were of the NIV genotype, and *F. graminearum* s. str. strains were of the 15-ADON genotype. An exception was the *F. cortaderiae* strains from maize that were either of the NIV or 3-ADON genotype (Table 2.1).

Trichothecene genotypes were also identified in a collection of 202 FGSC strains from maize stalks collected in two states, Goiás and Paraná, showing the high prevalence (96 %) of the NIV genotype compared to the 15-ADON (3 %) and 3-ADON (1 %) genotypes (Ciliato 2012). A study with 115 FGSC strains obtained from soybean seeds from both Central and South Brazil also reported that the NIV

genotype was more frequent (55 %), with a higher frequency of the 15-ADON (42 %) and a very low frequency of the 3-ADON (3 %) genotypes (Alves 2012). These strains are yet to be identified to the phylogenetic species level.

2.5 Fitness-Related and Aggressiveness Traits

Strains representative of the FGSC populations from Brazil have been examined with regard to asexual (germination, mycelial growth and sporulation) and sexual (perithecia and ascospores formation) reproductive and pathogenicity traits in a small number of studies. In an early study by Rivadenera (2001), 85 *Fusarium* spp. isolates showed variation in relation to their mycelial growth rate and pathogenicity, a result that could be due to not all of the isolates belonging to the FGSC complex or to different species of FGSC that had not yet been identified at the time. A few isolates that were not obtained from wheat heads did not produce perithecia, in contrast to the putative FGSC isolates that produced abundant perithecia in less than 2 weeks in carnation leaf agar. For the three groups discriminated by their mycelial growth rate in PDA under dark conditions, most isolates grew an average of 1.7 cm/day and 1.38 cm/day, whereas a few isolates grew at a rate of 2.26 cm/day (Rivadenera 2001).

More recently, isolates representative of different phylogenetic species of the complex and obtained from different hosts have been studied comparatively with regard to fitness-related traits and pathogenicity. Using two pairs of FGSC isolates obtained from wheat and barley, each belonging to *F. graminearum* s. str. or *F. meridionale*, Spolti et al. (2012a) examined the mycelial growth rate, sporulation and germination rates, sensitivity to tebuconazole and aggressiveness of the isolates. The values of all the variables examined, excepting the germination rate, differed significantly between the two species: on average, the *Fusarium graminearum* s. str. isolates grew 1.3× faster, sporulated 3× more, and exhibited lower sensitivity to tebuconazole than the *F. meridionale* isolates. In a pathogenicity assay based on *in vitro* glume infection and *in planta* central-floret inoculation methods, the *F. graminearum* s. str. isolates were capable of infecting and colonizing at higher rates than the *F. meridionale* isolates, a result only observed for the less susceptible (cv. Guamirim) and not the more susceptible (cv. BRS 194) variety. The authors discussed that such differences may explain the greater predominance of *F. graminearum* s. str. in wheat, as previously observed (Scoz et al. 2009; Astolfi et al. 2012b).

The aggressiveness toward maize seedlings of two *F. meridionale* and two *F. graminearum* s. str. obtained from maize kernels was evaluated using a substrate-inoculation method. Although no significant difference was found between the species, a large variation was observed for plant height (18.6–20.5 cm) and dry weight (1.8–2.3 g) (Silva 2011). In another study, strains of these two species were also compared in relation to their aggressiveness by the inoculation of maize stalks at 60 days after emergence, but no significant difference was found (Ciliato 2012). Lastly, the mycelial growth and aggressiveness of these two species was compared

based on the inoculation of stalks of 21-day-old seedlings (Kuhnem et al. 2013). Contrary to a previous study (Spolti et al. 2012a) that tested strains from wheat and barley, the mycelial growth rate did not differ significantly between these species from maize, which may be due to the low number of isolates studied and the large variation of isolates from the same species (Kuhnem et al. 2013). Furthermore, all the isolates were pathogenic toward the stalks of maize seedlings (21 days old) using a toothpick-inoculation method. Although the *F. graminearum* s. str. strains produced lesions that were, on average, 10 mm larger than those produced by *F. meridionale* at 14 days after inoculation, no significant difference was found when the isolates were grouped by species, in agreement with two other studies (Silva 2011; Ciliato 2012). Such behavior may be due to the greater variability between the isolates (6.3–53.5 mm) and, as in the previous study with isolates from wheat, also suggests that a higher sample size is necessary to better determine the potential differences in aggressiveness between these species.

2.6 Fungicide Sensitivity

In Brazil and in other regions of the world, fungicides are becoming a key component in integrated FHB management (Casa et al. 2007; Spolti et al. 2013). Chemical control has been used more intensively since the early 1990s when FHB epidemics became more frequent and research data showed the economic benefits of the use of fungicides in some situations (Reis et al. 1996; Picinini and Fernandes 1998). Triazol fungicides, such as tebuconazole and metconazole, are those most recommended, though commercial mixtures of triazol and strobilurins are also labeled and used in Brazil. In Europe and China, declines in fungicide sensitivity and increases in resistance have been detected and are suspected to be due to the intensive use of fungicides (Klix et al. 2007; Yin et al. 2009). However, the information in the literature was incomplete with regard to triazol fungicide sensitivity at the FGSC population level in Brazil until Spolti et al. (2012b) observed variability in the levels of sensitivity in 50 FGSC strains, with EC_{50} (effective concentration at which 50 % of mycelial growth is reduced) median values of 0.001 and 0.037 mg/L for tebuconazole and metconazole, respectively. Additionally, cross-resistance was detected, but no fitness-related cost was identified based on the fact that the less and more resistant groups of strains did not differ in their mycelial growth rates. In a previous study in which the sensitivity to tebuconazole of only four isolates was examined, two *F. meridionale* isolates exhibited a higher sensitivity than *F. graminearum* s. str. (Spolti et al. 2012b). Further preliminary data from the comparison of 10 strains from each species showed that the EC_{50} values of the *F. graminearum* s. str. strains were an average of 4.5-fold higher than *F. meridionale* (Spolti et al. 2013). Although variation in sensitivity levels has been observed when comparing these two species in different experiments, possibly due to a low sample size or other factors, *F. meridionale* has consistently shown a higher sensitivity to triazol in comparison to *F. graminearum* s. str. strains.

Another small-scale study about fungicide sensitivity was conducted using five FGSC strains obtained from either maize stalk or wheat and barley grains grown in PR and RS states, but that were not identified to the FGSC species level (Avozani et al. 2011). Results showed significant variation among the strains with regards to EC_{50} values, which were relatively low for triazoles (<0.01 for metconazole, prochloraz, prothioconazole and tebuconazole and 0.12 for cyproconazole) and benzimidazole (0.07 for carbendazim) fungicides and higher for strobilurin (0.21 for azoxystrobin and 1.33 for trifloxystrobin) fungicides.

2.7 Conclusions

As in other regions of the world, plant disease epidemics and the contamination of cereal grains as a result of infection with FGSC have received considerable attention in recent years by Brazilian researchers working alone or in collaboration with researchers from abroad. Advances in global research and the availability of novel tools to facilitate a thorough examination of the genotypic variability of the populations are leading to a better characterization of the regional populations and understanding of risk factors that were heretofore unknown.

The results found to date through molecular surveys conducted in important cereal crops indicate that South-Central Brazil is one of the few regions in the world that possesses an exceptionally great diversity of FGSC species. Additionally, there is evidence that fixation for specific tricothecene genotypes has occurred in some species, in contrast with other regions where there appears to be much less species diversity but the presence of dominant species possessing a variety of tricothecene genotypes. Moreover, we have not found evidence in surveys conducted over 5 years of any potential shift toward any specific tricothecene genotype or species of the complex, as reported in other regions. In fact, surveys on multiple cereals have indicated that the species may be more adapted to specific hosts or niches, which deserves further investigation. For example, it is not known why the composition and frequency of species change dramatically from one crop to another in the same growing region, e.g., wheat and maize. This observation may be due to a true host preference or some bias in the sampling either with regard to the number of samples or the niche from which the samples are obtained.

A better understanding of the relationship between the tricothecene genotypes and chemotypes is needed to understand why, for example, the NIV mycotoxin is found in grains at concentrations that does not reflect the frequency of the NIV genotypes found in symptomatic kernels. This could be related either to the ability of the populations to produce a single tricothecene, as expected from the genotypic analysis, or to differences in species compositions, which can vary from field to field or due to selection pressure by management practices or the environment. Further investigations on the exploration of phenotypic traits that include pathogenicity, fitness and fungicide sensitivity will help in the understanding of and propose hypotheses about the ecology of the pathogen and the epidemiology of the diseases

caused by the *F. graminearum* complex in Brazil. The recent promulgation of tolerance limits for *Fusarium* mycotoxins in several cereal crops in Brazil will surely contribute to increased awareness and the consideration of *F. graminearum* as a major threat to cereal production and public health, a problem that will only be solved by continued monitoring and multi-disciplinary research.

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