Regional and Field-Specific Factors Affect the Composition of Fusarium Head Blight Pathogens in Subtropical No-Till Wheat Agroecosystem of Brazil

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ABSTRACT

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A multiyear survey of >200 wheat fields in Paraná (PR) and Rio Grande do Sul (RS) states was conducted to assess the extent and distribution of *Fusarium graminearum* species complex (FGSC) diversity in the southern Brazilian wheat agroecosystem. Five species and three trichothecene genotypes were found among 671 FGSC isolates from Fusarium head blight (FHB)-infected wheat heads: *F. graminearum* (83%) of the 15-acetyldeoxynivalenol (15-ADON) genotype, *F. meridionale* (12.8%) and *F. asiaticum* (0.4%) of the nivalenol (NIV) genotype, and *F. cortaderiae* (2.5%) and *F. austroamericanum* (0.9%) with either the NIV or the 3-ADON genotype. Regional differences in FGSC composition were observed, with *F. meridionale* and the NIV type being significantly ($P < 0.001$) more prevalent in PR (>28%) than in RS ($\leq 9\%$). Within RS, *F. graminearum* was overrepresented in fields below 600 m in elevation and in fields with higher levels of FHB incidence ($P < 0.05$).

Species composition was not significantly influenced by previous crop or the stage of grain development at sampling. Habitat-specific differences in FGSC composition were evaluated in three fields by characterizing a total of 189 isolates collected from corn stubble, air above the wheat canopy, and symptomatic wheat kernels. Significant differences in FGSC composition were observed among these habitats ($P < 0.001$). Most strikingly, *F. meridionale* and *F. cortaderiae* of the NIV genotype accounted for the vast majority (>96%) of isolates from corn stubble, whereas *F. graminearum* with the 15-ADON genotype was dominant (>84%) among isolates from diseased wheat kernels. Potential differences in pathogenic fitness on wheat were also suggested by a greenhouse competitiveness assay in which *F. graminearum* was recovered at much higher frequency (>90%) than *F. meridionale* from four wheat varieties inoculated with an equal mixture of *F. graminearum* and *F. meridionale* isolates. Taken together, the data presented here suggest that FGSC composition and, consequently, the trichothecene contamination in wheat grown in southern Brazil is influenced by host adaptation and pathogenic fitness. Evidence that *F. meridionale* and *F. cortaderiae* with the NIV genotype are regionally significant contributors to FHB may have significant implications for food safety and the economics of cereal production.

Fusarium head blight (FHB) is a fungal disease of major economic concern to small grain production worldwide, particularly due to contamination of grain with dangerous mycotoxins produced by the fungus during pathogenesis (15,25). The *Fusarium graminearum* species complex (FGSC), which comprises at least 16 recognized species (31,32,46,52,62), is the primary cause of FHB in many parts of the world. Among members of the FGSC, *F. graminearum* is the most prevalent species, causing FHB in many parts of the world, and is particularly dominant among isolates from wheat in Europe (55,61) and the Americas (3,42,59). In South America, six other species within the FGSC have been found affecting wheat and other cereal crops grown in Brazil, Argentina, and Uruguay, including *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. brasiliense*, *F. cortaderiae*, and *F. meridionale* (2,3,32,44,49,56).

FGSC species are known to produce several mycotoxins but special attention has been given to trichothecenes, which are harmful for both human and animal health (11). Deoxynivalenol (DON) is the most significant among the trichothecenes, given its

widespread occurrence in wheat grain at levels of concern. Due to the difficulties associated with the preharvest management of FHB, several countries promulgated maximum limits for mycotoxin contamination in grains and foods, especially for DON (57). However, nivalenol (NIV), another trichothecene mycotoxin, has been observed at equal or higher concentrations than DON in the Netherlands, Japan, and Brazil (10,53,54).

Three strain-specific profiles of trichothecene production, or chemotypes, have been identified among members of the FGSC, including NIV and its acetyl-derivatives, DON and primarily 3-acetyldeoxynivalenol (3-DON), and DON and primarily 15-ADON (26). Trichothecene chemotype diversity has been maintained by selection, indicating that chemotype differences can have important consequences for the fitness of the producing organism (58). The genetic basis for chemotype differences has also been identified (1,20,22), and several polymerase chain reaction (PCR)-based assays are available for genetic determination of the trichothecene chemotype potential of individual isolates (7,39,58,59). In addition, a multilocus genotyping (MLGT) assay based on interrogation of single-nucleotide polymorphisms located in several housekeeping genes and genes involved in trichothecene biosynthesis has been developed for simultaneous determination of species identity and trichothecene genotype (31,46,59,62). Although inconsistencies between the trichothecene

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cene genotype and the chemotype of some isolates have been infrequently reported (28,45), these molecular assays constitute useful tools for mapping trichothecene genotype distributions and conducting large-scale molecular surveillance of FGSC composition (2,33,36,40,42,49,59).

In Brazil, two molecular surveys for the identification of FGSC and trichothecene genotypes have been conducted in wheat. The first analyzed a collection of 82 isolates obtained from *Fusarium*-damaged kernels harvested across three states (49) and the second analyzed three collections of ≈ 50 isolates each from three individual fields (3). Both studies showed the presence of only *F. graminearum* and *F. meridionale*, although four other species of the complex have been documented in Brazilian isolates collected from small grain cereals and soybean seed (2,23,32).

The main objectives of the present study were to expand knowledge of the species composition and toxigenic potential of FHB pathogens in southern Brazil using a much larger collection of FHB isolates and to improve understanding of the factors affecting species composition based on both field survey and experimental data. An additional objective was to evaluate differences in species composition from collections of isolates obtained from within-field corn residues, the air above the wheat canopy, and infected wheat kernels. These field survey and experimental data were used to test our hypotheses that the FGSC composition on wheat in southern Brazil is (i) more diverse than previously documented, (ii) influenced by region- or field-specific factors in no-tilled wheat crops following soybean or corn, and (iii) influenced by differences in ecology or pathogenic fitness on wheat among members of the FGSC.

MATERIALS AND METHODS

FHB survey and regional collection of isolates. Isolates that composed the regional collection were obtained from symptomatic wheat spikes collected during visits to commercial wheat fields in Rio Grande do Sul (RS) and Paraná State (PR), Brazil. In RS, we visited fields from the major wheat-growing region in the northern portion of the state during October and November of three consecutive cropping years (2009 to 2011). From 49 to 60 fields were assessed per year, totaling 160 fields (Fig. 1). In each field, 20 sampling units separated by ≈ 10 m were randomly assigned following a W-shape sampling design. In each sampling unit, 10 spikes from contiguous main tillers were sampled. Crop developmental stage according to Zadoks scale (63) at sampling, previous summer crop (corn or soybean) based on visible crop stubble, latitude and longitude coordinates, and the elevation above sea level was recorded for each of these fields. In the laboratory, each spike was rated visually for FHB incidence (spike is symptomatic or not) and severity (proportion of diseased spikelets per total spikelets). In PR, commercial wheat fields were visited from July to August during 2009 and 2011. In 2009, the samplings were made in both northern and southern locations at 25 municipalities. In 2011, all isolates were obtained from three municipalities in the southern region of PR state (Fig. 1).

Analyses of habitat-specific differences in FGSC composition. Three field settings were established in the northern region of RS state during 2011. They were named Santa Bárbara (SBA), Marau (MAR), and Vacaria (VAC), based on the municipality in which they were located. These fields were 240 to 260 km apart and at similar latitude (latitude 28.3 to 28.5) but varying longitude, SBA being the western-most location (longitude -53.25), MAR being centrally located (longitude -52.78), and VAC in the eastern portion (longitude -50.93) of the state. The fields were nontilled and had visible corn stubble at the soil surface. Two wheat cultivars, 'BRS Guamirim' and 'Quartzo', which are known as moderately susceptible and susceptible to FHB, respectively, were sown. FHB intensity was assessed 3 to 4 weeks after flowering and 50 wheat spikes were randomly collected at the

three central rows of each of the four plots. The spikes were taken to the laboratory, where visual assessments of FHB incidence and severity were made. The plots were hand harvested in an area of 5 m² and threshed mechanically using seed cleaning equipment, as described elsewhere (51). The incidence of *Fusarium*-damaged kernels (FDK, %) (small and shrunken kernels with or without pink or white mycelia) was assessed visually in at least 100 mature kernels.

The collection of isolates in each field was made at three distinct habitats related to different stages in the life cycle of the fungus: corn stubble (survival stage), air above the canopy (dispersal stage), and infected kernel (infection and colonization stages). These collections are referred to as saprophytic, airborne, and pathogenic, respectively.

The saprophytic collection was obtained during visits to the fields during the stem elongation stage of crop development. In all, ≈ 50 segments of corn stubble exhibiting black-colored perithecia were collected per field. The airborne collection was composed of fungal isolates taken from FGSC-like colonies grown in petri plates containing Nash-Snyder medium (6) mounted on stationary spore traps that were positioned 0.5 m above the canopy (9,47). The samplings were made during five consecutive nights when the crop was at the full-flowering stage. On each night, five plates were placed at 1700 h and collected at 0900 h. After each collection period, the plates were covered with a lid and returned to the laboratory for incubation. To compose the pathogenic collection, *Fusarium*-damaged mature kernels from the bulk of harvested kernels from the plots were carefully selected, surface disinfested, and incubated on potato-dextrose agar (PDA) medium.

Fig. 1. Geographic locations of (i) commercial wheat fields surveyed for *Fusarium* head blight incidence, severity, and *Fusarium graminearum* species complex (FGSC) isolates in Rio Grande do Sul (RS) state from 2009 to 2011 and (ii) municipalities where commercial wheat fields were surveyed for FGSC isolates during 2009 and 2011 in Paraná (PR) state.

Isolation and purification procedures. The isolates from the regional collection were obtained from symptomatic spikelets that were excised and incubated under wet conditions inside a plastic box containing a wet blotter paper. After 7 to 10 days, isolations were made from colonies resembling FGSC. A single isolate was obtained from one spike. For the saprophytic collection of isolates, fragments of corn stubble were immersed in 0.2% sodium hypochlorite for 30 s, rinsed three times in sterile deionized water, blotted dry on sterile filter paper, and stored at 4°C until processing. Prior to processing, small segments were surface disinfected in 70% ethanol for 30 s and 0.2% sodium hypochlorite for 30 s, then rinsed three times in sterile deionized water. Under the microscope, a single perithecium was removed from the segment and placed on PDA plates containing streptomycin at 40 mg/liter (37). For the airborne collection, plates containing the selective media exposed to the air above the canopy were incubated for 5 to 7 days at room temperature (22°C) under ambient light conditions. Salmon-colored colonies that developed on the selective media were subcultured onto fresh plates of PDA (47). Finally, the pathogenic collection of isolates was obtained from typical FGSC colonies grown from *Fusarium*-damaged kernels plated on PDA. All isolates were purified following a single-spore subculture and were prepared for long-term preservation as described previously (3).

Genomic DNA extraction. Prior to DNA extraction, the purified isolates were grown on a PDA plate for 7 days and the mycelia were removed, dried in filter paper, and stored at 4°C. Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) protocol with some modifications, as described previously (3). The mycelia were macerated in liquid nitrogen and transferred to microtubes (1.5 ml) where 750 µl of CTAB (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl [pH 8], and 20 mM EDTA [pH 8]) and 15 µl of β-mercaptoethanol were added. Tubes were incubated for 15 min at 65°C, and 500 µl of chloroform/isoamyl alcohol (24:1) solution was used for extraction. After centrifuging at 11,000 rpm for 10 min, 500 µl of the supernatant were transferred to another microtube, and 5 µl of RNase suspension at 20 mg/ml was added. Tubes were incubated in hot-tube at 37°C for 30 min. DNA was precipitated with 500 µl of isopropanol, and 250 µl of 7.5 M ammonium acetate was added. After centrifuging at 11,000 rpm for 10 min, the supernatant was discarded and the pellet washed off in 100 µl of 70% ethanol solution. The pellet was resuspended in 100 µl of Tris-EDTA (10 mM Tris-HCl [pH 7.5] and 0.1 mM EDTA).

Species and trichothecene genotype identification. Most isolates were simultaneously assigned to an FGSC species and trichothecene genotype via MLGT using a Luminex 100 flow cytometer, as described previously (59). The MLGT assay was based on allele-specific primer extension using a 48-probe set (46,59,62) targeting species and trichothecene chemotype-specific genetic variation in six genes. A subset of isolates were identified to species based on the analysis of partial sequences of the transcription elongation factor 1α gene (30) and further sequence similarity searches with BLAST network service of the Fusarium ID database (<http://isolate.fusariumdb.org/blast.php>) (14,35). The trichothecene genotypes of those isolates were determined via multiplex PCR assays targeting portions of the *Tri3* and *Tri12* genes (58,59) under the conditions described previously (3).

Interspecies competition pathogenicity assay. Four wheat varieties varying in FHB resistance were used: ‘BRS 194’, classified as susceptible; ‘BRS Guamirim’, classified as a moderately susceptible; ‘Frontana’, classified as a moderately resistant; and ‘Sumai 3’, classified as resistant to FHB. Seed were sown in pots (10 seeds per 5-liter pot) containing substrate (equal parts of humus, soil, and vermiculite) fertilized with macronutrients (N-P-K at 10:10:10). Pots were maintained in the greenhouse from planting to flowering. Mean daily temperature was 25°C, mean minimum temperature was 20°C, and mean relative humidity was 60%

in the greenhouse during the experiment. The plants were spray inoculated with a macroconidial suspension (1×10^5 spores/ml) at early-milk grain stage (8). The inoculum was composed of four isolates: two *F. graminearum* and two *F. meridionale* that had been previously characterized as highly pathogenic (50). The final concentration was adjusted (vol/vol) for equal concentration of each isolate (1:1:1:1). After inoculation, plants were individually covered with a plastic bag and incubated at ≈25°C during 48 h in a growth chamber. Afterward, the bag was removed and the plants were moved back into the greenhouse. Assessment of disease severity was made at the 16th day after inoculation. The experiment was in a randomized complete block design with three replicates, a replicate being composed of potted plants with 10 spikes. At kernel maturity, grains were hand harvested and hand threshed, and FDK were surface disinfested and incubated on PDA medium. The recovered isolates were single spored and identified to one of the four inoculated isolates based on a vegetative compatibility assay (24). For such, four mycelial plugs of a distinct recovered isolate were placed equidistantly around the outer edge of a petri dish filled with V8-wheat medium (150 ml of V8 juice, 20 g of ground wheat seed, and 20 g of agar per 1 liter of medium) (24). A mycelial plug of the inoculated isolate was placed at the center of the plates, which were incubated at room temperature (22°C) and continuous light. After 10 days, the presence of barrage zones at the junctions of growing colonies was indicative of an incompatible reaction. Our previous experiments showed that the inoculated isolates always showed absence or presence of barrage zones when paired with its clone or cultures of the other isolate, respectively. The experiment was repeated once. Results were similar between the experiments and the data from the second experiment are presented.

Data analyses. The χ^2 test ($P < 0.05$) was used to evaluate differences in species composition in relation to year, state, and categorical field-specific factors for fields from RS state: field elevation above sea level, previous crop, grain stage at sampling, and FHB incidence. Also, the χ^2 test and Fisher’s exact test (for small sample size) were used to compare frequencies of the species grouped by prevalence (most dominant, second in dominance, and others) among the three habitats of all fields combined and frequencies of the species among the fields for all habitats combined. Finally, the same test was used to evaluate differences in the frequencies of *F. graminearum* and *F. meridionale* isolates recovered from spikes that were inoculated with an equal mixture of isolates of both species in a competitiveness assay.

RESULTS

Species and trichothecene genotypes in the regional collection. Five species within the FGSC were found in the regional collection of 671 isolates, in order of prevalence: *F. graminearum* (83%), *F. meridionale* (12.8%), *F. cortaderiae* (2.5%), *F. austroamericanum* (0.9%), and *F. asiaticum* (0.4%) (Table 1). The two most common species (*F. graminearum* and *F. meridionale*) were widely distributed across the surveyed production regions (Fig. 2). Two of the less frequent (<3%) species, *F. asiaticum* and *F. cortaderiae*, were found only in RS state, and *F. austroamericanum* was found in both states in similarly low frequencies (Fig. 2).

All three trichothecene genotypes were observed: 15-ADON was the most common (83.1%), followed by NIV (15.4%) and 3-ADON (1.34%). 15-ADON and NIV isolates were observed in each of the annual collections from both PR and RS states, whereas 3-ADON isolates were observed only during 2010 in RS state and 2011 in PR state (Fig. 2).

The variation in the trichothecene genotype of the isolates was highly structured in relation to species differences (Fig. 2). All 557 *F. graminearum* isolates were of the 15-ADON trichothecene genotype (Fgrr15A) and all but one of the 86 *F. meridionale*

isolates were of the NIV genotype (FmerNIV) (Fig. 2). The identification of a single 15-ADON *F. meridionale* isolate was confirmed following independent culturing and DNA extraction. The three *F. asiaticum* isolates were of the NIV genotype. Isolates with either NIV or 3-ADON genotypes were observed within *F. cortaderiae* (FcorNIV and Fcor3A) and *F. austroamericanum*, with NIV predominating (82.4%) among *F. cortaderiae* isolates and 3-ADON being more common (75%) among *F. austroamericanum* isolates. *F. austroamericanum* isolates of the NIV genotype were found only in PR State (Fig. 2).

The relative frequencies of *F. meridionale* and the NIV genotype were significantly ($P < 0.0001$) higher in PR State (28.2 and 29.2%, respectively) compared with RS State (5.5 and 9% respectively), with corresponding differences in the frequencies of *F. graminearum* and the 15-ADON genotype (Table 1; Figs. 2 and 3). Within each state, species composition did not change significantly across the sampled years ($P > 0.17$).

Exclusively for the collection of isolates from RS, where field-specific data were available, the χ^2 tests showed weak evidence for the effect of FHB incidence ($\chi^2 = 6.23$, $P = 0.044$) (Fig. 4D) and field elevation above the sea level ($\chi^2 = 6.11$, $P = 0.046$) (Fig. 4A) on FGSC composition. *F. graminearum* was overrepresented in fields below 600 m in elevation and in fields with higher levels of FHB incidence ($P < 0.05$). However, there was no evidence that species composition was influenced by previous crop ($\chi^2 = 1.92$, $P = 0.38$) (Fig. 4B) or the stage of grain development at sampling ($\chi^2 = 2.93$, $P = 0.23$) (Fig. 4C).

Disease assessment and species composition in within-field collections. FHB epidemics developed naturally in the three field settings (SBA, MAR, and VAC). FHB incidence was higher in MAR and VAC fields than in SBA but FHB severity was similar among these fields, averaging $\approx 7\%$ (Table 2). Visibly damaged kernels were generally rare ($< 2\%$) and were observed at lower frequency in VAC than in the other two fields (Table 2). In total, 189 isolates were obtained from all fields combined. The pathogenic collection accounted for $\approx 50\%$ of the isolates, whereas the saprophytic and airborne collections accounted for 28.5 and 17.9% of the isolates, respectively (Table 2).

Across the three fields, FGSC composition in the pathogenic collection was similar to that of the regional survey in RS state. However, species composition differed across the three habitats according to χ^2 tests ($\chi^2 = 107.48$, $P = < 0.001$) and Fisher's test for use with small sample sizes ($P < 0.001$). Among the 101 isolates in the pathogenic collection, *F. graminearum* with the 15-ADON genotype was dominant (84.2%) and *F. meridionale* (NIV) was found in low frequency (5.0%). *F. cortaderiae* (NIV) was found only in diseased kernels of the VAC field and in greater frequency than FmerNIV (Fig. 5C). In the saprophytic population, FmerNIV (68.5%) and FcorNIV (27.8%) predominated and only one Fgra15A was observed. Whereas FmerNIV was the dominant type collected from corn stubble of the MAR (western) and SBA fields (central) (Fig. 5A and B), FcorNIV was the most dominant type found in corn stubble of the VAC field (eastern) (Fig. 5C). FGSC composition in the airborne collection more closely

TABLE 1. Frequencies (%) of species and trichothecene genotypes among 671 *Fusarium graminearum* species complex (FGSC) isolates obtained from a regional collection of symptomatic wheat spikes from fields in Paraná state (PR) and Rio Grande do Sul state (RS) of Brazil

Species, genotype	PR state (%)			RS state (%)				Overall (%)
	2009	2011	All years	2009	2010	2011	All years	
FGSC								
<i>F. graminearum</i>	74.8	62.4	69.4	93.4	86.6	89.1	89.5	83.0
<i>F. meridionale</i>	25.2	32.3	28.2	4.1	6.0	6.5	5.5	12.8
Other ^a	0.0	5.4	2.3	2.0	7.4	4.3	5.1	3.8
Trichothecene genotype ^b								
Deoxynivalenol	74.8	65.6	70.8	93.9	89.8	89.1	91.0	84.5
Nivalenol	25.2	34.4	29.2	6.1	10.2	10.9	9.0	15.4
Subtotal (%)	18.3	13.8	32.1	21.9	32.1	13.7	67.8	100.0
Subtotal (number)	123	93	216	147	216	92	455	671

^a Other species of the FGSC in small frequencies includes *F. asiaticum* ($n = 3$), *F. austroamericanum* ($n = 8$), and *F. cortaderiae* ($n = 17$).

^b Deoxynivalenol is the combined frequency of 15-acetyldeoxynivalenol (15-ADON) and 3-ADON isolates.

Fig. 2. Number and geographical location of five species of the *Fusarium graminearum* species complex (Fasi = *F. asiaticum*, Faus = *F. austroamericanum*, Fcor = *F. cortaderiae*, Fgra = *F. graminearum*, and Fmer = *F. meridionale*) and their respective trichothecene genotypes (3-acetyldeoxynivalenol [3-ADON], 15-ADON, and nivalenol [NIV]) in a regional population of 671 isolates obtained from symptomatic wheat spikes from fields of Rio Grande do Sul state (southern) and Paraná state (northern).

matched the pathogenic populations from each of the individual fields. However, the overall composition of the airborne collection was intermediate between that of the pathogenic and saprophytic collections, with Fgra15A accounting for 67.6% of the isolates and FmerNIV and FcorNIV observed at 17.6 and 14.7% frequencies, respectively.

Interspecies competitiveness assay. In order to assess the relative pathogenic fitness of the two most common FGSC types encountered in our surveys, potted wheat plants were inoculated during flowering with an equal mixture of Fgra15A and FmerNIV isolates. FHB symptoms were found in most (>94%) of the inoculated spikes of all cultivars, with the exception of Sumai 3, which

Fig. 3. Mosaic plots for the proportion of isolates of the two most dominant species (Fgra = *F. graminearum*; Fmer = *F. meridionale*) and their respective trichothecene genotype (15A = 15-acetyldeoxynivalenol [ADON], 3A = 3-ADON, and NIV = nivalenol) and other less frequently found species and genotypes (*F. asiaticum* NIV, *F. austroamericanum* 3A, *F. austroamericanum* NIV, *F. cortaderiae* 3A, and *F. cortaderiae* NIV) obtained from symptomatic spikes in wheat fields sampled in different states.

Fig. 4. Mosaic plots for the proportion of isolates of the two most dominant species (Fgra = *F. graminearum* and Fmer = *F. meridionale*) and their respective trichothecene genotype (15A = 15-acetyldeoxynivalenol [ADON], 3A = 3-ADON, and NIV = nivalenol) and other less frequently found species and genotypes (*F. asiaticum* NIV, *F. austroamericanum* 3A, *F. austroamericanum* NIV, *F. cortaderiae* 3A, and *F. cortaderiae* NIV) obtained from symptomatic spikes in wheat fields sampled in fields that varied in **A**, elevation; **B**, crop residue from summer crop; **C**, grain stage during sampling; and **D**, Fusarium head blight incidence.

showed only 1% of inoculated spikes with symptoms. FHB severity was recorded in the three other varieties and the mean values corresponded to their FHB susceptibility levels (BRS 194 = 75%, Guamirim = 52%, and Frontana = 44%). Similarly, *Fusarium*-damaged kernel incidence values were, on average, 74, 57, and 48% for BRS 194, Guamirim, and Frontana, respectively.

In total, 39 isolates were recovered from damaged kernels of each cultivar, with the exception of Sumai 3, which also had no visible FHB symptoms in the kernels. In total, 12 isolates were recovered each from BRS 194 and Frontana and 15 isolates from BRS Guamirim. Most isolates (89.7%) were of the Fgra15A type and four isolates of the FmerNIV type were recovered only from BRS Guamirim. The recovery frequency differed significantly between these two species across all cultivars ($\chi^2 = 7.68$, $P = 0.021$).

DISCUSSION

In a previous study, we documented the predominance of Fgra15A causing FHB of wheat in southern Brazil and identified FmerNIV at lower frequency (3,49). In the current study, we analyzed a much larger collection of FGSC isolates, the largest to our knowledge in South America, by using a structured sampling of isolates in fields from multiple years and two states, different regions within the states, and several fields within regions. Additionally, isolates were collected from distinct habitats related to the stages of the fungus life cycle. With this approach, we expanded considerably our knowledge of the diversity and spatial distribution of species and trichothecene genotypes of the FGSC in the main subtropical nontilled wheat agroecosystems of Brazil.

TABLE 2. Summary of disease assessment scores and number of isolates obtained for each of three sampled habitats representing stages in the life cycle of the fungus in three fields of the northern Rio Grande do Sul state, Brazil

Disease, collection	Fields			Sum/mean
	Santa Bárbara (west)	Marau (central)	Vacaria (east)	
FHB intensity (%) ^a				
Incidence	14.8 ± 4.2	29.3 ± 11.8	24.7 ± 6.3	22.9
Severity	9.3 ± 3.4	6.7 ± 2.8	5.2 ± 2.4	7.1
FDK	1.7 ± 0.5	1.6 ± 0.4	0.7 ± 0.4	1.2
Number of isolates/collection ^b				
Saprophytic	17	20	17	54
Airborne	6	19	9	34
Pathogenic	27	40	34	101
Sum	50	79	54	189

^a Mean ± standard deviation for *Fusarium* head blight (FHB) incidence (percentage of symptomatic spikes) and severity (percentage of diseased spikelets per spike) measurements in 200 wheat spikes from eight noninoculated plots, and *Fusarium*-damaged kernels (FDK) assessed in 100 kernels from eight non-inoculated replicates.

^b Within-field collections from sampling at different habitats representing stages in the life-cycle of the fungus: saprophytic (survival stage) obtained from perithecial isolations from corn stubble 1 month prior to flowering, airborne (dispersal stage) from the air above the canopy during flowering, and pathogenic (infection and colonization stage) from *Fusarium*-damaged mature kernels at harvest.

Fig. 5. Mosaic plots summarizing relative frequencies of *Fusarium cortaderiae* of the nivalenol (NIV) genotype (FcorNIV), *F. graminearum* of the 15-acetyldeoxynivalenol (15-ADON) genotype (Fgra15A), and *F. meridionale* of the NIV genotype (FmerNIV) in three fields from western, central, and eastern portions of the northern Rio Grande do Sul State Brazil. In total, 186 isolates were obtained from the three fields combined. In each field, isolates were collected at three habitats, thus composing the following collections: saprophytic, from perithecial isolations from corn stubbles collected around 30 days prior to flowering; airborne, from collections of viable airborne spores using a selective media on traps placed above canopy during flowering; and pathogenic, from isolations from symptomatic mature kernels prior to harvest.

The expanded survey data confirmed the predominance of *F. graminearum* and demonstrated that *F. meridionale* is also an important regional contributor to FHB of wheat in southern Brazil. Moreover, we provided the first documentation of the relative frequency of three additional FGSC species associated with FHB symptoms in wheat crops of the country. *F. austroamericanum*, *F. asiaticum*, and *F. cortaderiae*, which were absent from our previous analyses, were found at low frequencies (<3%) in the total sample. However, the frequency of *F. cortaderiae* in RS state was similar to that of *F. meridionale*. Moreover, in our previous survey, we had a very small sample size from the northeastern portion of RS state, where most of the *F. cortaderiae* isolates were encountered in the current study. Hence, it is difficult to hypothesize whether *F. cortaderiae* was present or absent or has increased in frequency since our previous survey in RS state.

F. graminearum likely evolved in North America but this species has a global distribution and is a common cause of FHB in South America (32,40,56). *F. meridionale*, *F. austroamericanum*, and *F. cortaderiae* were initially described from South American isolates and are part of a phylogenetic clade that likely originated in South America (32,46). However, *F. asiaticum* is hypothesized to be Asian endemic and has only recently been identified as a causative agent of FHB of wheat in South America (56). Although all of these three additional FGSC species encountered in this study were reported previously from random collections in Brazil, no information was available for their year of sampling, location, and relative frequency in wheat (23,32).

F. graminearum was the only member of the FGSC observed in association with FHB of wheat in Argentina, and was also found to be the most common member of the FGSC on wheat in Uruguay (40,56). As in the current study, *F. asiaticum*, *F. cortaderiae*, and *F. austroamericanum* were recently found in Uruguayan wheat. However, *F. meridionale* was not observed among isolates from wheat collected in Argentina or Uruguay, despite our data indicating that *F. meridionale* is a substantial contributor to FHB of wheat in Brazil, especially in the state of Paraná.

In line with our previous findings and those of a number of recent regional surveys (3,5,21,56,64), trichothecene genotype variation was highly structured in relation to species limits. Hence, differences in trichothecene genotype composition were also apparent. NIV accounted for 15.4 and 13.2% of the isolates obtained from wheat in Brazil and Uruguay, respectively, but was not observed among FGSC isolates from wheat in Argentina. In Brazil, the frequency of the NIV genotype is primarily due to *F. meridionale*, while *F. asiaticum* was the most common species with the NIV genotype found in Uruguay. Taken together, these data indicate that, although Fgra15A is predominant across major wheat-growing areas in South America, FGSC species composition on wheat is different in Brazil, Argentina, and Uruguay.

We found that FmerNIV was present at a much higher frequency in PR state (28.2% on average) compared with RS state (5.5% on average), with a corresponding difference in the frequency of the NIV genotype (29.2 and 9.0%, respectively). The basis for this significant regional difference in FGSC composition is not clear but may be related to differences in the relative prevalence of maize production in these two states. Previously, Sampietro and collaborators (44) reported that FmerNIV accounted for 84.8% of the FGSC isolates from maize in Argentina and *F. boothii* accounted for the rest of the maize isolates, whereas Fgra15A was the only member of the FGSC isolated from wheat in Argentina (40). Sampietro and collaborators (44) interpreted this host-specific difference in pathogen composition as suggestive of a difference in host adaptation, with *F. meridionale* and, possibly, *F. boothii* being better adapted to colonization of maize in comparison with *F. graminearum*. A large survey of FGSC

diversity in South Africa also identified significant differences in FGSC composition between isolates from FHB-infected heads of wheat in which *F. graminearum* predominated and that of maize ears in which *F. boothii* was dominant and *F. graminearum* was absent (5). Based on these data and previously published reports of FGSC diversity on maize, those authors hypothesized that *F. graminearum* may not be the primary FGSC species associated with ear rot of maize worldwide, and that *F. graminearum* may not be as adapted to colonization of maize ears as were other members of the FGSC (5). The acreage of maize crops in PR was estimated to be ≈2.5 million ha in 2011, which is two times higher than the acreage of maize grown in RS state for the same year (17). The possibility to grow maize twice in a year in PR may favor the build-up of *F. meridionale* populations available to infect wheat, which may partially explain the higher frequency of *F. meridionale* in PR state.

In addition to significant regional differences in FGSC composition, we observed significant habitat-specific differences in species composition within individual fields sampled in RS state. The most striking result was the uncoupling of saprophytic (maize stubble) and pathogenic (infected kernel) populations that were constituted mainly of FmerNIV/FcorNIV and Fgra15A, respectively. As in the regional survey, Fgra15A was the predominant species isolated from symptomatic kernels in all three fields. However, FmerNIV and FcorNIV accounted for 96.3% of the isolates obtained from corn stubble in those fields. We speculate that compositional differences among the habitats in the same field also may be reflective of species-specific adaptation to different hosts or environments. The saprophytic population from corn stubble likely reflects the pathogenic population most associated with maize, as suggested (44), but may also reflect a difference in saprophytic fitness favoring FmerNIV and FcorNIV over Fgra15A. Conversely, previous studies have documented that *F. graminearum* can be more aggressive on wheat than some other members of the FGSC (16,50,51,56). In addition, a previous study showed evidence that species and trichothecene type influenced the relative aggressiveness of different strains toward wheat, with *F. graminearum* and the 15-ADON type being associated with higher aggressiveness relative to *F. asiaticum* or *F. graminearum* with the NIV type (13). In the present study, we extended this evidence of differences in relative aggressiveness by demonstrating that Fgra15A isolates were much more competitive on wheat than FmerNIV isolates, as shown by the recovery of the former type at significantly higher frequencies following inoculation of wheat cultivars with an equal mixture of isolates representing the two FGSC types.

The observed differences between the saprophytic and pathogenic isolates also suggest that within-field inoculum from stubble may contribute less to FHB in a particular field than the airborne population derived from both within-field and external sources of inocula. The airborne collection from three fields that were separated by an average of 250 km had a composition of FGSC species and trichothecene genotypes that was intermediate between the pathogenic and saprophytic populations, though more similar to that seen in the pathogenic population. This is consistent with our finding that FGSC composition was not significantly influenced by the previous crop (corn or soybean) observed in an individual field. In some regions of the world, the presence of large amounts of within-field corn stubble is considered an important risk factor for FHB epidemics, especially in temperate climates where the residue decomposition rate is lower than in the subtropics (12,37). In Brazil, virtually all fields are grown under no-till, where abundant small fragments of crop stubble can serve as sources of inoculum. However, numerous alternative hosts are available year round, so that the pathogen population is able to persist in a subtropical climate environment without the limitations imposed by the cold winters of the temperate regions. As such, airborne inoculum of *F. graminearum*

had been commonly found year round in the wheat agroecosystem in southern Brazil (9,29,34) even before the widespread adoption of no-till in the mid 1980s (41). Studies on the relative importance of different inoculum sources for FHB epidemics in the United States have suggested that background inoculum (i.e., atmospheric settling and washout originating from distant sources) may be a much stronger contributor to FHB infections (19). In fact, the ability for the FHB pathogen to disperse long distances has been proven since viable inoculum of FHB pathogen was found at up to 320 m above the crop canopy (48) and infecting wheat from 750 m to many km from a known source (18). The uniqueness of cropping and climate conditions in Brazil may contribute to the increased diversity of species and genotypes affecting wheat, and may also reduce the relative importance of within-field stubble as a primary source of inoculum.

We found weak evidence that species frequencies were affected by categories of field elevation (600-m threshold), which was a surrogate variable for temperature. Temperature was previously reported to affect the relative frequencies of *F. graminearum* and *F. asiaticum* in China (38), and differences in trichothecene genotype composition were associated with elevation above sea level along the Yangtze River (60). However, physical barriers to the spread of a putative introduced population were hypothesized to explain the effect of elevation studies which reported that temperature differences were not a significant factor behind the regional differences in FGSC composition observed in China (60,64). Climate modeling based on observed distributions indicated that there were few climatic limits on the distribution of *F. graminearum* within the major rain-fed regions of wheat production (4), and the results of a previous study based on controlled-environment data indicated that mycelial growth of *F. graminearum* was equal to or greater than that of *F. meridionale* over a wide range of temperatures (50). Our results indicate that elevation and temperature may influence FGSC composition on wheat in southern Brazil but regional differences in crop rotations and species-specific differences in relative aggressiveness toward wheat likely play a larger role. We also found some evidence that *F. graminearum* prevalence increased with higher FHB incidence scores. However, the effect was modest and consistent with evidence that *F. graminearum* is a more aggressive pathogen of wheat (50), resulting in greater damage to wheat kernels than is observed for *F. meridionale* (51).

In summary, in this study, we have documented the prevalence and distribution of five FGSC species and their trichothecene genotypes associated with FHB of wheat in southern Brazil, identified significant regional differences in FGSC species and trichothecene genotype composition, determined that *F. graminearum* is more competitive on wheat than *F. meridionale*, and demonstrated that FGSC composition differs significantly among different habitats within individual fields. Taken together, the data presented here suggest that host preference and ecological niche adaptation may regulate FGSC composition and toxin contamination in wheat grown in southern Brazil. Evidence that *F. meridionale* and *F. cortaderiae* with the NIV genotype are regionally significant contributors to FHB may have significant implications for food safety and the economics of cereal production, because nivalenol has been reported to have higher vertebrate toxicity than DON (27,43). As a result of the potential differences in toxicity, the European Scientific Committee on Food has proposed a lower tolerable daily intake limit for NIV (0.7 µg/kg of body weight) than for DON (1 µg/kg of body weight) (57). Although maize as the previous crop in an individual field did not influence FGSC composition on wheat, we hypothesize that regional differences in maize production may have a significant influence on pathogen composition and, thus, NIV contamination levels. This indicates the need for analyses of FGSC composition on maize and additional regional surveys of trichothecene contamination levels in grains from Brazil.

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