Nationwide survey reveals high diversity of *Fusarium* species and related mycotoxins in Brazilian rice: 2014 and 2015 harvests

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ABSTRACT

Ninety three samples of rice were obtained from research and commercial plots at eight rice-producing regions of Brazil and analyzed for the presence of *Fusarium* species and 14 mycotoxins. A total of 352 isolates belonging to *Fusarium* genus were obtained from 85 % of the samples. These were assigned to four species complexes (SC) based on morphological identification. The most frequent SC detected was *F. incarnatum-equiseti* (FIESC, 32.4 %) followed by *F. fujikuroi* (FFSC, 26.1 %), *F. graminearum* (FGSC, 24.7 %) and *F. chlamydosporum* (FCSC, 16.8 %). FGSC was limited geographically and dominant in the southern subtropical production regions while the others occurred in all regions, particularly FIESC, the most widespread among them. The samples were individually contaminated with three to eight mycotoxins. The most common mycotoxins detected were zearalenone (ZEA), beauvericin, and acetylated forms of deoxynivalenol (AcDON). Other toxins included enniatins, T-2, HT-2, DON, neosolaniol and moniliformin. The concentration levels were all below the Brazilian promulgated limits established only for DON (< 750 ppb), and ZEA (< 100 ppb) with one exception for the latter. Most toxins were found in both the husk and flour fractions, but AcDON tended to concentrate more in the husk. Our survey extends considerable our knowledge of the *Fusarium* complexes infecting rice and provides an update on the status of rice mycotoxin contamination at the country level, which can be considered generally safe. However, attention should be paid to the widespread contamination of beauvericin.

Keywords:

Oryza sativa; Beauvericin; Deoxynivalenol; Fumonisin; Trichothecenes; Zearalenone.

Highlights

- Four species complex (SC) of *Fusarium* were found in 93 samples of Brazilian rice
- *F. incarnatum-equiseti* SC, *F. Fujikuroi* SC, and *F. graminearum* SC were predominant
- Three to eight *Fusarium* mycotoxins contaminated the samples
- Zearalenone, beavericin and acetylates of deoxynvalenol were most widespread
- The concentration of the regulated mycotoxins were below the Brazilian legislation

1. Introduction

Rice is one of the world's most important staple food crops produced mainly in Asia (AMIS, 2019). Elsewhere in the world, Brazil is the largest rice-producing country contributing 12 million tons harvested yearly which is destined mainly for domestic consumption (FAO, 2018). The lowland subtropical regions in the south of Brazil accounts for approximately 80 % of rice production under flooding conditions, but the crop is also cultivated in the northern and midwestern tropical regions, under both rainfed (upland) and flooding (lowland) conditions (FAO, 2019).

Several *Fusarium* species that are commonly found in association with rice kernels are capable of producing a wide range of mycotoxins that are harmful to human and animal health (Savi et al., 2018; Ok et al., 2014; Park, Choi, Hwang, & Kim, 2005; Desjardins et al., 2000). Commonly, these fungi are isolated from plants or panicles with no apparent disease symptoms or abnormality. A couple species are known to produce symptoms on seedlings or panicles. A seedling disease is called bakanae, caused by *F. fujikuroi*, a member of *F. fujikuroi* species complex (FFSC) (Amatulli, Spadaro, Gullino, & Garibaldi, 2010; Wulff et al., 2010). Infected seedlings may exhibit abnormal elongation and, in severe cases, stunting leading to death. Although reported

in Brazil during the late 1960s (Amaral, Regina, Ferreira, & Fazio, 1970), bakanae is not of concern to rice production in the country. However, toxigenic species within FFSC are commonly reported infecting rice kernels, thus raising concerns about the presence of fumonisins, the main mycotoxin produced by isolates of this species complex (Savi et al., 2018; Wulff et al., 2010; Desjardins et al., 2000).

Occasionally, panicles in the field appear blighted due to infection by species of the *F. graminearum* species complex (FGSC), the main cause of Fusarium head blight of wheat and barley and Gibberella ear rot (GER) of maize (Kuhnem et al., 2016; Del Ponte et al., 2015). Symptoms of panicle blight appear sporadically in the south of Brazil and these have been reproduced in a controlled environment when panicles were challenged with FGSC inoculum (Scheidt et al., 2019). FGSC species produce mainly B-trichothecene (deoxynivalenol, nivalenol and their acetylates) and zearalenone mycotoxins (O'Donnell, Kistler, Tacke, & Casper, 2000). A few FGSC species, which varied in their toxigenic profile for trichothecene production, were found infecting rice in Rio Grande do Sul state, Brazil (Gomes, Ward, Badiale-Furlong, & Del Ponte, 2015). The most dominant species detected in that study was *F. asiaticum*, a nivalenol-producing species commonly associated with head blight of small grains in Asia. A more recent survey identified *F. graminearum* (but not *F. asiaticum*), together with *F. verticillioides*, in rice samples collected in Brazil (Savi et al., 2018). Although FGSC is not commonly reported as a pathogen of rice in Brazil, its widespread occurrence in kernels warns for the risk of mycotoxin contamination (Savi et al., 2018; Gomes, Ward, Badiale-Furlong, & Del Ponte, 2015).

The most common targeted mycotoxins in rice grain are fumonisins, trichothecenes and zearalenone, but attention should be given to mycotoxins of relatively lower toxicity such as beauvericin, moniliformin, enniatins and fusaproliferin given the large

diversity of mycotoxigenic *Fusarium* species that infect cereal crops and their “emergence” in recent surveys (Marin, Ramos, Cano-Sancho, & Sanchis, 2013; Savi et al., 2018). In Brazil, the health regulatory agency has promulgated maximum tolerated limits for deoxynivalenol (DON) and zearalenone (ZEA) in raw cereal grains and byproducts, including rice (ANVISA, 2011, 2017). In fact, fumonisin B1 (FB1), DON and ZEA are more commonly analyzed than other *Fusarium* toxins in recent surveys around the world, including Brazil, but their presence and concentration vary across regions and years, and are usually limited to detected the regulated mycotoxins (Almeida et al., 2012; Petrarca, Rossi, & de Sylos, 2016; Savi et al., 2018). The main objectives of this study were to determine the occurrence and prevalence of mycotoxigenic *Fusarium* and to detect and quantify more than a dozen *Fusarium*-related mycotoxins in rice grain samples from the main rice-producing regions of Brazil during two harvest seasons.

2. Materials and methods

2.1. Study area and sampling

Ninety three rice kernel samples were obtained from 30 locations across eight Brazilian states during two growing seasons: 2014/15 and 2015/16. The number of samples varied among the states as well number and spatial coverage survey within each state. The most represented state, with regards to the number of samples, was Goiás (GO), where 29 samples were obtained, but all samples were originated from a single experimental site. Rio Grande do Sul (RS) state was represented by 28 samples obtained at 16 different fields. The other third of the samples were obtained from fields in Mato Grosso (MT), Santa Catarina (SC), Tocantins (TO), São Paulo (SP), Maranhão (MA) and Roraima (RR) (Fig. 1).

The origin and sampling time relative to harvest varied across the states. Samples from RS, SC and TO were obtained from visits to rice fields prior to harvest. During each visit, 20 sampling units (SU) were randomly defined during a walk in the field (10 m apart) and ten rice panicles were sampled per SU, totaling 200 panicles per field. These panicles were dried, stored in a paper bag and brought to the laboratory where they were preserved until performing the fungal isolations.

2.2. *Fungal isolation and identification*

Subsamples of 200 seeds were subjected to a standard rice seed health blotter test (Misra, Merca, & Mew 1994). After disinfestation, 50 seeds were arranged per plastic box (10 × 10 cm) containing three papers moistened with 6% saline solution to prevent seed germination. The boxes were incubated at 25 °C and 12 h photoperiod for a period of 12 days. Thereafter, seeds were visually inspected under a stereoscope microscope for evaluating fungal growth. Random samples of fungi depicting typical and distinct *Fusarium* morphology were isolated on potato dextrose agar (PDA, Acumedia, Neogen Corporation). The colonies were purified on synthetic nutrient-poor agar (SNA, Nirenberg, 1976) incubated for 11-12 days at 25 °C, and 12 h light/dark cycle. The isolates were assigned to species complex level according to morphological features of the colony, conidiogenous cells and conidia (Leslie & Summerell, 2006).

2.3. *Mycotoxins and kernel fractions*

Fourteen mycotoxins were analyzed and grouped into seven classes: A-trichothecene (T-2 toxin, HT-2 toxin and neosolaniol [NEO]), B-trichothecene (deoxynivalenol [DON] and its acetylated derivatives [sum of 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol]), zearalenone (ZEA), moniliformin (MON), fumonisins

(FB1 and FB2), beauvericin (BEA) and enniatins (ENN A, ENN A1, ENN B, ENN B1). The detection and quantification were performed for each sample twice due to their separation into husk and flour substrates.

2.3.1. Sample preparation protocol for mycotoxin analysis

Samples were prepared following Rodríguez-Carrasco, Moltó, Berrada, & Mañes (2014). The extract obtained was evaporated to dryness under a gentle nitrogen flow at 45 °C and the residue reconstituted with a mixture of MeOH:water (70:30, v/v), which was filtered (0.22 µm) prior to analysis.

2.3.2. Analysis by UHPLC-Q-Orbitrap HRMS

Analyses were performed using an UHPLC instrument (Dionex Ultimate 3000, Thermo Fisher Scientific, Waltham, Ma, USA) coupled to a Q Exactive Orbitrap mass spectrometer (UHPLC, Thermo Fischer Scientific, Waltham, Ma, USA). The UHPLC system consisted of a degassing system, a Quaternary UHPLC pump working at 1250 bar, an autosampler device and a thermostated Luna Omega column (50 × 2.1 mm, 1.6 µm, Phenomenex) held at 30 °C. The mobile phases were: phase A, water with 0.1 % formic acid and 5 mM ammonium formate; and phase B, MeOH with 0.1 % formic acid and 5 mM ammonium formate. A linear gradient elution program was applied as follows: initial 0 % B was held for 1 min and then increased to 95 % B in 1 min and held for 0.5 min. Then, the gradient was linearly decreased to 75 % in 2.5 min and decreased again to 60 % B in 1 min. After that, gradient was reduced to 0 % in 0.5 min and held for 1.5 min for re-equilibration, giving a total run time of 8 min. The flow rate was 0.4 mL/min and the injection volume 5 µL.

Detection was performed using a Q Exactive mass spectrometer. The mass spectrometer was operated in both positive and negative ion mode by setting two scan events (Full ion MS and All ion fragmentation, AIF). Full scan data were acquired at a resolving power of 35000 FWHM at m/z 200. The conditions in positive ionization mode (ESI+) were: spray voltage 4 kV; capillary temperature 290 °C; S-lens RF level 50; sheath gas pressure ($N_2 > 95\%$) 35, auxiliary gas ($N_2 > 95\%$) 10, and auxiliary gas heater temperature 305 °C. Ion source parameters in negative (ESI-) mode were: spray voltage -4 kV; capillary temperature 290 °C; S-lens RF level 50; sheath gas pressure ($N_2 > 95\%$) 35, auxiliary gas ($N_2 > 95\%$) 10, and auxiliary gas heater temperature 305 °C. Value for automatic gain control (AGC) target was set at 1×10^6 , a scan range of m/z 100-1000 was selected and the injection time was set to 200 ms. Scan-rate was set at 2 scans/s.

The parameters in the positive ion mode for the scan event of AIF were as follows: mass resolving power = 17500 FWHM; maximum injection time = 200 ms; scan time = 0.10 s; ACG target = 1×10^5 ; scan range = 100 – 1000 m/z . The conditions in negative ion mode for the scan event of AIF (ESI-) were: mass resolving power = 17500 FWHM; maximum injection time = 200 ms; scan time = 0.10 s; ACG target = 1×10^5 ; scan range = 100 – 1000 m/z . Data analysis and processing were evaluated by the Quan/Qual Browser Xcalibur software v. 3.1.66 (Xcalibur, Thermo Fisher Scientific).

3. Results

3.1. Incidence of the Fusarium species complexes

A total of 352 isolates with characteristics of the genus *Fusarium* were obtained from 85 % (79/93) of the kernel samples. The number of isolates per sample ranged

from one to 29 (one panicle sample from a field in Itajaí, SC), but most of the samples contributed less than five isolates (Fig. 2A). The majority of the isolates were obtained from three states (SC, GO, RS) that also contributed more samples. The other five states contributed less than 40 isolates each (Fig. 2C).

Isolates belonged to four distinct species complexes. The most represented (about a third of the isolates) was *F. incarnatum-equiseti* species complex (FIESC), followed by FFSC and FGSC at similar frequencies. The least frequent was *F. chlamydosporum* species complex (FCSC) (Fig. 2B). As to geographical distribution, FIESC was found in all states; FFSC was not recovered from SP; and FCSC was not found in MA and RR. FGSC was isolated from samples of three states: RS, SC and TO (Fig. 2C).

The occurrence of one species complex in a sample dominated (35/93), but multiple occurrences of two, three and four complexes were also noted (Fig. 3A). In the single occurrence, the dominant species was FIESC (18/35) followed by FCSC (11/35), FFSC (5/35) and FGSC (1/35). The frequencies of the species complexes were more similar for the multiple occurrences. The higher the number of isolates obtained from sample, the more frequent the multiple occurrences of three and four complexes (not shown). When at least two species complexes were found in a sample, the pairwise combination showed that FIESC and FCSS or FFSC appeared together more frequently (Fig. 3B).

3.2. Mycotoxin prevalence and concentration levels

All samples, considering the two substrates, were contaminated with at least three and a maximum of eight mycotoxins with concentration >LOQ (Fig. 4A). The mycotoxins were found in both flour and husk, with a few differences in the relative

concentration depending on the mycotoxin. There was a general tendency for the husk substrate to have more samples with concentrations <LOQ (Table 1). The most prevalent mycotoxins (> 70 % of the samples) were AcDON, ZEA, BEA and HT2, in this order. NEO and the other toxins appeared in less than 30 and 15 %, respectively (Fig. 4B). Considering both fractions, the concentration levels were mostly below the 10 ppm for all toxins. There were exceptionally higher concentration of HT-2, BEA and ZEA in the flour fraction but no greater than 811 ppb (Table 1).

3.3. Geographical distribution of the mycotoxins

The five most prevalent mycotoxins were found in all or more than half of the samples collected in the eight Brazilian states (Fig. 4B). The highest BEA and ZEA values were found in samples from MT state (Fig. 5). The AcDON tended to concentrate more in the husk than in the flour, regardless of the origin of the samples (Fig. 5). The opposite was noted for ZEA and DON, but the trend was not consistent across the states. DON was more common in RS state at higher levels in the flour than in the husk (Fig. 5).

4. Discussion

In this study we gathered and analyzed the most comprehensive data on the presence and geographic distribution of *Fusarium* species complex and quantification of 14 *Fusarium* mycotoxins in rice from the main production regions of Brazil. Surveys of mycotoxin in Brazilian rice have been conducted for several decades but mainly targeting those produced by other fungal genera (*Aspergillus*, *Penicillium*) (Rodríguez-Amaya & Sabino, 2002; Almeida et al., 2012; Katsurayama et al., 2018). Increasing attention has been given to *Fusarium* mycotoxins after the establishment of maximum

tolerated limits for mycotoxins in Brazilian cereals during the early 2010s. *Fusarium* species have been reported more recently associated with different rice production systems in Brazil (Katsurayama et al., 2019), but taxonomic studies on the diversity of *Fusarium* in Brazilian rice are scarce and limited to small sample, therefore the main species and their frequencies in the country as a whole have been largely unknown. Most routine seed pathology and rice grain contamination studies, which usually target identification of several fungi in the sample, have reported *Fusarium* at the genus level based on colony morphology and quick assessment of conidial structures.

We were unable to detect *Fusarium* in 15 % of the samples and one to two isolates were obtained from around half of the *Fusarium*-infected samples that presented low incidence. Based on *Fusarium* identification using morphology, we could segregate 352 isolates into four species complexes. When at least two species co-occurred in the sample (half of the samples), we found FIESC was the most common pair of FFSC or FCSC. Increased diversity of *Fusarium* spp. infecting rice kernels has also been shown in previous studies worldwide (Gonçalves et al., 2019, Amatulli, Spadaro, Gullino, & Garibaldi, 2010; Desjardins et al., 2000). A Korean study reported species of the same four complexes reported here infecting white and brown rice, with FGSC being the most frequent (Ok et al., 2014). In another Korean study, FFSC was dominant (16 % of samples), followed by FGSC (10 %) and FIESC (7 %) in samples of polished rice from grain wholesale markets (Park, Choi, Hwang, & Kim, 2005). FFSC have been isolated from both asymptomatic and symptomatic plants for bakanae in Africa and Asia (Wulff et al., 2010), Italy (Amatulli, Spadaro, Gullino, & Garibaldi, 2010; Dal Prá, Tonti, Pancaldi, Nipoti, & Alberti, 2010) and Malaysian Peninsula (Hsuan, Salleh, & Zakaria, 2011). Differences in species composition between studies may be due to sampling, target organ (plant or seed), geographical distribution of the pathogen, climate effect of

species distribution etc. In Brazil, while FIESC and FFSC were present in most regions, FGSC was limited to the subtropics where small grains are more commonly grown and act as important host for FGSC that causes Fusarium head blight (Del Ponte et al., 2015).

In a previous study by our group, we obtained 130 rice samples from RS state and isolated FGSC from the samples based on morphology. Their further identification to species made using a sophisticated multilocus molecular assay allowed to determine *F. asiaticum* as the dominant species but others were also important contributors within FGSC including *F. meridionale*, *F. graminearum* and *F. cortaderiae*, which were all pathogenic to rice panicles (Gomes, Ward, Badiale-Furlong, & Del Ponte, 2015). In the current survey, we were interested in expanding our knowledge of the presence of FGSC in other states, beyond southern Brazil, and compare with other *Fusarium* species complexes. Our new data show that FGSC isolates were the most frequent *Fusarium* from SC and RS states, where this complex mostly occurred in the country with the exception of two isolates from TO state in the Northeast of Brazil. Our results agrees with a recent study that showed the dominance of FGSC (*F. graminearum* and *F. asiaticum*, seven isolates each) among 20 isolates from RS state, and its absence among 14 isolates from MA state where FIESC (11/14) was dominant (Katsurayama et al., 2019).

Another recent survey with 100 samples of rice (several varieties and fresh, dried or parboiled) obtained from southern Brazil in 2017 harvest season, FGSC and FFSC isolates were both found at significant level in dried samples (14/25), while FIESC and *F. culmorum* were rarely found (1 or 2/25) in the freshly harvested samples (Savi et al. 2018). The sequencing of RPB2 gene of four FGSC and four FFSC isolates the identification of only *F. graminearum* and *F. verticillioides*. These results are in

contrast with our hypothesis that *F. asiaticum* is the most FGSC species in Brazilian rice (Gomes, Ward, Badiale-Furlong, & Del Ponte, 2015). The difference between the studies may be due to the extension of the sampled regions and years. Studies are underway in our group to confirm the hypothesis of *F. asiaticum* as the main FGSC species associated with rice in Brazil. Moreover, while *F. verticillioides* was the only FFSC species reported by Savi et al. (2018), our further identifications of FFSC isolates showed that several other species within this complex were also detected, reflecting a much larger diversity of FFSC members occurring on rice (Nicolli et al., unpublished). Similarly, 70 isolates obtained from the same survey, which were identified morphologically as FIESC, were separated into nine phylogenetic species of FIESC (Avila et al., 2019).

We found FCSC as the least frequent complex in our survey, agreeing with report in India where it was the least frequent morphological species occurring in unsterilised seeds (Islam & Borthakur, 2012), but contrasting with Saudi Arabia, where it appeared as the most prevalent among other fusaria (Kanaan & Bahkali, 1993). In Brazil, FCSC members were recently reported associated with rice produced in upland regions in central and north of Brazil (Katsurayama et al., 2019). Further studies are underway in our group to accurately identify isolates of the FCSC complex detected in our survey as well as their toxigenic potential (Gomes et al., unpublished). There are evidences that FCSC can produce B-trichothecenes, as this species complex is nested within a larger group of trichothecene producing species (Moretti, Susca, Mulé, Logrieco, & Proctor, 2013; O'Donnell et al., 2013; Gomes et al., unpublished), but the mycotoxin production needs confirmation.

The samples were contaminated mostly with five different mycotoxins (maximum of eight), which is in agreement with previous studies targeting multiple toxin

contamination. In Ivory Coast, up to eight mycotoxins were detected in more than 40 samples of imported or local rice (Manizan et al., 2018). The presence of several toxigenic species, as shown in our study, increases the risk of multiple toxin contamination as well as potentiate the concentration of specific toxins that are produced by more than one species (Gonçalves et al., 2019). For example, members of FGSC earlier isolated from Brazilian rice (Gomes, Ward, Badiale-Furlong, & Del Ponte, 2015) and the FIESC obtained in our survey (Avila et al., 2019) have been confirmed as producers of DON, nivalenol and ZEA, which might explain partially the widespread occurrence of AcDON and ZEA. In fact, DON was found predominantly in the samples from RS and SC states where FGSC are common. Further work should also target the presence of nivalenol, a mycotoxin that was not analysed in our survey, and the one that has shown to be produced by Brazilian *F. asiaticum*, the most dominant FGSC species in Brazilian rice (Gomes, Ward, Badiale-Furlong, & Del Ponte, 2015).

The regulated mycotoxins in Brazilian rice (DON and ZEA) were at levels mostly below legislation (ANVISA, 2011, 2017). The exception was one sample contaminated with ZEA above the limit of 100 ppb for processed rice. No sample showed DON above the limits (750 ppb). We found other mycotoxins that are not regulated in Brazil and worldwide, such as AcDON at higher concentration than DON, which tended to concentrate in the husk rather than the flour. This is a critical information for the industry because of the migration into the internal portion of the grain that can occur during some processes such as rice parboiling (Schaarschmidt & Fauhl-Hasek, 2018; Dors, Pinto, & Badiale-Furlong, 2009). Studies have shown similar effects DON and AcDON alone or in combination on human cells (Juan-García, Taroncher, Font, & Ruiz, 2018). Continuous survey of mycotoxin contamination in rice is also important for assuring the quality of Brazilian product. Brazil has exported ~590 thousand tons of rice

to Venezuela, and smaller amounts to Senegal, Nicaragua, Cuba, Costa Rica and the United States (USDA, 2019). In Venezuela (5 ppb) and Peru (10 ppb) mycotoxin limits are established for aflatoxin B1, B2, G1, G2 (FAO, 2004).

Fumonisin B1, B2 and B3 are produced by members of FFSC, mostly by *F. verticillioides* and *F. proliferatum*, and, at a lower level, by *F. fujikuroi* (Desjardins, 2006). The most prevalent species of FFSC in our survey was *F. fujikuroi* (Nicolli et al., unpublished) which might explain the low fumonisin levels in rice samples in Brazil. Members of FFSC can also produce BEA, MON and enniatins. These three mycotoxins are for the first time reported in Brazil, but have been found in rice elsewhere (Nazari, Sulyok, Kobarfard, Yazdanpanah, & Krska, 2015; Sifou et al., 2011) and they are considered emerging mycotoxins for which potential mycotoxin-specific effects have been reported in animals (Bertero, Moretti, Spicer, & Caloni, 2018). However, no regulated maximum limits have been determined yet, worldwide. In Europe, maximum limits of *Fusarium* mycotoxins were established for DON, ZEA and the sum of fumonisin B1 and B2, whereas for the T-2 and HT-2 toxins, only indicative levels for cereal products have been set (EFSA, 2014).

5. Conclusion and recommendations

Results of this survey extend considerably the knowledge of the *Fusarium* species complexes infecting rice and update on the status of contamination of rice with mycotoxins at the country level, which were generally at safe levels. Continuing surveys in rice for the assessment of mycotoxin contamination are important for assuring the quality of Brazilian rice for both domestic consumption and export.

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Table 1

Number of contaminated samples (prevalence) in 93 rice samples above and below the limit of quantification (LOQ) in both the husk and flour fractions and their respective concentrations determined by HPLC analysis. Samples were collected in eight states of Brazil during 2014/15 and 2015/16 growing seasons.

Mycotoxin ^a	Husk					Flour				
	Prevalence		Concentration (ppb)			Prevalence		Concentration (ppb)		
	No.	<LOQ ^b	Mean	Median	Max	No.	<LOQ	Mean	Median	Max
T-2	1	0	1.4	1.4	1.4	9	0	0.58	0.6	0.9
HT-2	61	11	2.3	1.7	11.2	36	8	1.55	1.3	3.7
NEO	24	53	0.89	0.75	2.2	4	20	0.9	0.85	1.2
DON	10	20	9.76	8.4	23.7	5	18	32.98	10.7	125.2
AcDON	91	0	51.53	43.5	156	66	0	3.76	2.7	17
ZEA	91	2	3.03	1.2	34.6	35	0	33.04	5.8	469.1
MON	1	2	13.3	13.3	13.3	4	4	9.7	4.4	28.1
FB1	0	1	<LOQ	<LOQ	<LOQ	1	6	4.1	4.1	4.1
FB2	3	12	2.83	2.9	3.4	1	5	2.1	2.1	2.1
BEA	61	21	9.71	2.9	110.4	81	5	23.33	6.1	810.1
ENN A	0	2	<LOQ	<LOQ	<LOQ	0	1	<LOQ	<LOQ	<LOQ
ENN A1	0	14	<LOQ	<LOQ	<LOQ	0	1	<LOQ	<LOQ	<LOQ
ENN B	1	1	2.6	2.6	2.6	0	6	<LOQ	<LOQ	<LOQ
ENN B1	2	0	0.85	0.85	1.2	2	5	1.45	1.45	2.4

^a Abbreviations: T-2 = toxin T-2; HT-2 = toxin HT-2; DON = deoxynivalenol; AcDON = sum of acetylated derivatives of DON: 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON); NEO = neosolaniol.

^b <LOQ = below the limit of quantification.

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622 **Fig. 1.** Geographical origin (dots on the map) and the number of rice samples (in 93 total) per
623 state (within parentheses in the legend) collected in eight rice-growing Brazilian states during
624 2014/15 and 2015/16 seasons. Cases of more than one sample per location are indicated with the
625 respective number next to the dot.

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Fig. 2. Frequency of *Fusarium* spp. isolates obtained per rice sample (A) which were assigned to four species complexes (SC), *F. chlamydosporum* (FCSC), *F. fujikuroi* (FFSC), *F. graminearum* (FGSC) and *F. incarnatum-equiseti* (FIESC) based on morphological characters, and varied in their frequency overall (B) as well as conditioned to each of the eight Brazilian states (SC = Santa Catarina; GO = Goiás; RS = Rio Grande do Sul; MT = Mato Grosso; MA = Maranhão; RR = Roraima and SP = São Paulo) (C). The number within parentheses for a state represent the number of rice samples.

Fig. 3. Number of co-occurrences (A) and frequency of pairwise co-occurrences (B) strains belonging to four species complexes (SC) - *F. chlamydosporum* (FCSC), *F. fujikuroi* (FFSC), *F. graminearum* (FGSC) and *F. incarnatum-equiseti* (FIESC) - in the same rice sample (panicle or kernel).

Fig. 4. Frequency of contaminated samples (A) and mycotoxins (B) found in 93 rice samples collected in eight Brazilian states during 2014/15 and 2015/16 growing seasons. Number within parentheses next to a state (color legend) represent the number of analyzed samples for the respective state.

Fig. 5. Distribution of concentration levels of beauvericin, deoxynivalenol (DON), acetylates of DON (AcDON), and zearalenone conditioned to each of eight Brazilian states (number of samples within parentheses) in samples collected in 2015 and 2016 harvest seasons.

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