Survey of freshly-harvested oat grains from Southern Brazil reveals high incidence of type B trichothecenes and associated *Fusarium* species

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Abstract

Oats are a nutrient rich cereal used for animal feed and growing in human consumption. This cereal can be affected by *Fusarium* spp., causing the disease Fusarium Head Blight. This disease is caused mainly by species within the *Fusarium graminearum* species complex, and are also responsible for producing mycotoxins that are harmful to humans and animals. This study aimed to investigate fungal diversity in Brazilian oat samples, focusing on the *Fusarium sambucinum* species complex and the presence of type B trichothecenes (deoxynivalenol and its derivatives, and nivalenol) from two different regions; Paraná (PR) and Rio Grande do Sul (RS). The isolated fungi from oat grains were identified as species from the genera: *Fusarium*, *Phoma* and *Alternaria*. The majority of *Fusarium* isolates belonged to the *Fusarium sambucinum* species complex; identified as *F. graminearum* s.s., *F. meridionale* and *F. poae*. In the RS region, *F. poae* was the most frequent fungus, while FGSC was the most frequent in the PR region. The majority of *F. graminearum s.s.* isolates were of the 15-ADON genotype, while some 3-ADON genotypes were identified; however, *F. meridionale* and *F. poae* were all of the NIV genotype. Mycotoxin analysis revealed that 92% and 100% of the samples from PR and RS were contaminated with type B trichothecenes, respectively. The oats from PR were predominantly contaminated with DON, whereas NIV was predominant in oats from RS. Analysis showed that 24% of the samples were contaminated with DON at levels higher than Brazilian regulations. Co-contamination of DON, its derivatives and NIV was observed in 84% and 57.7% of the samples from PR and RS, respectively. The results provide new information on *Fusarium* contamination in Brazilian oats, highlighting the importance for further studies on mycotoxins.

Keywords: Oats, *Fusarium sambucinum* species complex, deoxynivalenol, nivalenol, mycotoxin.

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1 Introduction

Oats (*Avena sativa* L.) have been consumed by humans and livestock since ancient times; it is considered a nutrient-rich cereal due to the high concentration of lipids, proteins, vitamins, antioxidants, minerals and β-glucan [1]. The global production of oats in 2020/2021 was 25,470 thousand metric tons, with the European Union being the largest producer; followed closely by Canada, Norway, Australia and Brazil [2].

Cereals can be affected by fungal diseases, which can lead to lower nutritional values and mycotoxin accumulation in the grains; resulting in reduced product quality and economic losses [3]. Numerous fungi may be attributed to various oat diseases; the *Fusarium* genus, however, is considered one of the major threats. One of the most serious and economically important diseases caused by the genus is Fusarium Head Blight (FHB), which affects cereal production worldwide [4-6].

Fusarium Head Blight is primarily caused by species in the *Fusarium graminearum* species complex (FGSC), however, other *Fusarium* species may also be involved. FHB causes flower abortion, and the formation of pitted, wrinkled and rough grains that are ‘pinkish’ in color [7]. Infection by these pathogens can also result in mycotoxin accumulation, mainly trichothecenes and zearalenone (ZEN) [8,9].

Trichothecenes produced by *Fusarium* species are classified into either type A or B; these compounds are differentiated by the C-8 function of the 12,13-epoxytrichothec-9-ene (EPT) core structure [10]. Members of the FGSC are able to produce type B trichothecenes, such as deoxynivalenol (DON) and its acetylated derivatives (3 acetyl-DON and 15 acetyl-DON; 3-ADON and 15-ADON); as well as nivalenol (NIV) and its acetylated forms [10].

In animals, DON has been linked to feed refusal, vomiting and weight reduction [11]. NIV can cause immunotoxicity and haematotoxicity, based on *in vitro* and *in vivo* tests [12]. The toxic effects of the acetylated DON forms are poorly documented; however, 15-ADON has been

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reported to be more toxic than DON and 3-ADON in *ex vivo* and *in vivo* tests using human intestinal cells and piglets [13]. Due to the toxic effects of DON in humans, a provisional maximum tolerable daily intake (PMTDI) of 1.0 μg/kg body weight/day has been set by the U.N. Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA) [14]. For NIV, a tolerable daily intake (TDI) of 1.2 μg/kg body weight/day has been set by the European Food Safety Authority (EFSA) [12].

Zearalenone is a cyclic compound containing a resorcyclic acid lactone structure; and is also primarily produced by the same fungi that produce type B trichothecenes. It is commonly found together with DON and NIV in cereals. ZEN is considered an estrogenic mycotoxin that causes abnormalities in the reproductive system, particularly in swine; leading to infertility, genital prolapse and enlarged mammary glands [15]. Due to these effects, the JECFA established a PMTDI of 0.5 μg/kg body weight/day [16].

In the Northern hemisphere, the main *Fusarium* species associated with oats are: *F. graminearum, F. avenaceum, F. sporotrichioides, F. langsethiae, F. poae, F. culmorum* and *F. tricinctum* [4-6,17-19]. This implies that a diverse range of mycotoxins may be found in oats. For example, *F. sporotrichioides* and *F. langsethiae* are responsible for T-2 and HT-2 (type A trichothecenes) accumulation in small grain cereals [20]; whereas *F. graminearum* and *F. culmorum* are able to produce ZEN and type B trichothecenes; *F. poae* mainly produces NIV [21]; and finally, *F. avenaceum* and *F. tricinctum* are able to produce other *Fusarium* mycotoxins, such as moniliformin (MON) and enniatins (ENNs) [22]. Indeed, several studies have already shown the occurrence of type A and type B trichothecenes in oats grown in colder climates [6,23-27].

In South America, a few studies have shown that *Alternaria, Aspergillus, Penicillium* and *Fusarium* are prevalent in oats [28-30]. Regarding the *Fusarium* genus, *F. graminearum, F. poae* and *F. verticillioides* have previously been recovered from freshly harvested grains [30].

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In regards to mycotoxin contamination, aflatoxin B1 (AFB1), DON, fumonisin B1 (FB1) and ochratoxin A (OTA) have been detected in oat grains and products [28,30-34].

In Brazil, reports of fungi and mycotoxin contamination in oats are scarce, with a few only focusing either on the mycobiota or mycotoxin contamination. The majority of the associated fungi recovered were *Alternaria, Drechslera*, *Fusarium* and *Puccinia* [28,29,35]. However, the only reported mycotoxin was FB1, while aflatoxins, ochratoxin A and ZEN were not detected [28,32].

Due to the lack of information on fungal diversity and mycotoxin contamination in Brazilian oats, together with the increasing production of this cereal within the country, the objectives of the current study were: i.) to characterize *Fusarium* species associated with freshly harvested Brazilian oats, ii.) to determine the levels of deoxynivalenol, its derivatives (3-acetildeoxynivalenol and 15-acetildeoxynivalenol) and nivalenol in the grain samples.

2 Results

2.1 Water activity and mycobiota of freshly harvested oat grains

Ninety-two percent of the oat samples were contaminated with fungi, predominantly by the *Fusarium* genus. In the RS region, samples were contaminated with *Fusarium*, followed by *Phoma*, *Epicoccum*, *Alternaria*, *Cladosporium*, *Penicillium*, *Aspergillus*, *Drechslera*, *Pestalotiopsis*, *Mucor*, *Rhizopus*, *Curvularia* and *Trichoderma*. Water activity (a_w) ranged from 0.4 to 0.6 (mean=0.54). No correlation between a_w and the occurrence of *Fusarium* was observed ($p<0.05$). In the PR region, 33.1% of the samples were contaminated with *Fusarium*, followed by *Alternaria*, *Nigrospora*, *Epicoccum*, *Phoma*, *Cladosporium*, *Rhizopus*, *Penicillium*, *Dreschelera*, *Mucor* and *Pestalotiopsis* (Table 1). Water activity ranged from 0.5 to 0.6 (mean=0.51). No correlation between a_w and the occurrence of *Fusarium* was observed ($p<0.05$).

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The *F. sambucinum* species complex (FSAMSC) was foremost in oat samples from both regions. In the RS region, *F. poae* was the primary species isolated, followed by the FGSC, *F. avenaceum* and *F. proliferatum.* In the PR region, the majority of the isolates belonged to the FGSC, followed by *F. poae*, *F. incarnatum-equiseti* species complex (FIESC), *F. verticillioides*, *F. subglutinans* and *F. solani* species complex.

Table 1. Frequency and mean count of fungal genera and *Fusarium* species complexes isolated from oat samples from two different regions of Brazil: Paraná (PR - 50 samples) and Rio Grande do Sul (RS - 50 samples).

Oat origin	**RS**	**PR**	**Average count (CFU/g)**
Oat a_w[a]	0.54	0.51	
Genera of fungi	**Frequency (%)**		
Fusarium	37.3	33.1	1.8×10^5
Phoma	15.4	11.1	7.7×10^4
Epicoccum	13.8	11.3	7.1×10^4
Alternaria	9.6	16.3	5.9×10^4
Cladosporium	7	6.9	3.3×10^4
Penicillium	4.4	1.9	2.2×10^4
Aspergillus	3.4	ND	2.3×10^4
Dreschlera	3	1.7	1.4×10^4
Pestalotiopsis	1.5	0.3	4×10^3
Mucor	1.5	1.7	7×10^3
Rhizopus	1.5	2.6	9×10^3
Curvularia	0.8	ND	6×10^3
Trichoderma	0.8	ND	6×10^3
Nigrospora	ND	13.1	4.3×10^4
***Fusarium* species complexes**	**Frequency (%)**		
FSAMSC	93.8	85.5	1.7×10^5
FTSC	3.2	0	7.6×10^3
FFSC	3	6.7	4.3×10^4
FIESC	ND	5	5.6×10^3
FSSC	ND	2.8	3.1×10^3

[a] Mean values for water activity; ND: not detected. FSAMSC: *Fusarium sambucinum* species complex; FTSC: *F. tricinctum* species complex; FFSC: *F. fujikuroi* species complex; FIESC: *F. incarnatum-equiseti* species complex; FSSC: *F. solani* species complex.

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2.2 Molecular characterization of *Fusarium* isolates

A sub-sample of *Fusarium* isolates were selected for molecular characterization. Isolates belonging to the FSAMSC were randomly selected for phylogenetic analysis of the second major subunit of the RNA polymerase locus (*RPB2*) and genotype characterization.

The phylogenetic analysis dataset consisted of 55 taxa, with 259 parsimony informative characters (PICs). The analysis resulted in one hundred most parsimonious trees (consistency index/CI = 0.76; retention index/RI = 0.95). No significant topological variations were detected between neighbor-joining, parsimony and likelihood phylogenies (data not shown). Most of the isolates were clustered within *F. graminearum* and *F. poae* species, and a few within *F. meridionale*. (**Figure 1**).

The FGSC isolates were predominantly 15-ADON (50%) genotype, followed by NIV (36.4%) and 3-ADON (13.6%). All strains identified as *F. meridionale* were characterized as NIV genotype, while the majority of *F. graminearum s.s* were characterized as 15-ADON and, in lesser frequency, as 3-ADON genotypes. As expected, all of the *F. poae* strains displayed a NIV genotype. The oat grain isolates from Rio Grande do Sul were identified mostly as NIV (75%) genotype, followed by 15-ADON (16.7%) and 3-ADON (8.3%). The isolates from Paraná mostly displayed the NIV genotype (70.4%), followed by 15-ADON (25.9%) and 3-ADON (3.7%).

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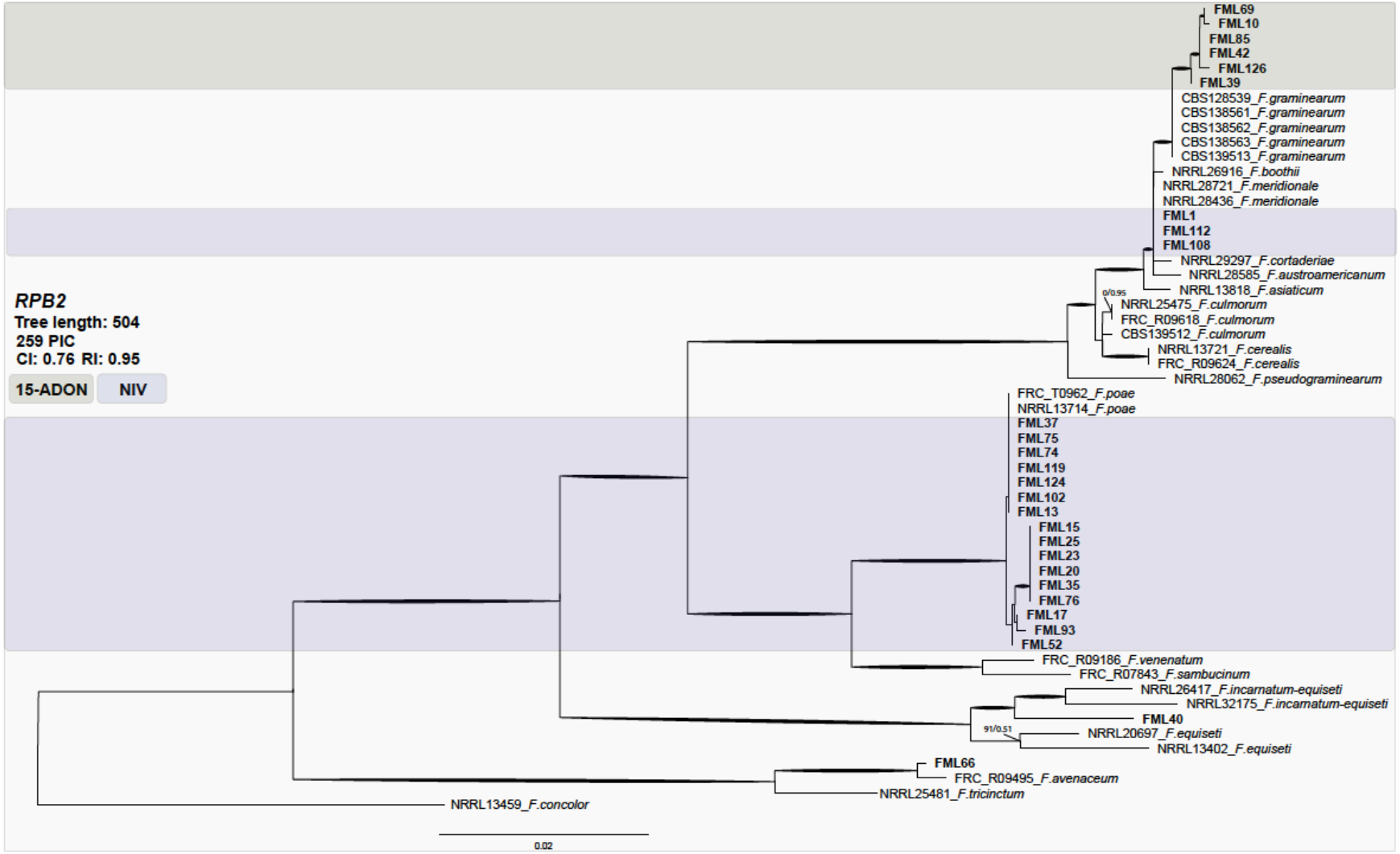
Figure 1. Maximum parsimony phylogeny inferred from the first fragment of the *RPB2 locus*. Bootstrap values above 70% and Bayesian posterior probabilities (BPP) above 0.9 are assigned in bold branches. Support values above branches are bootstrap/BPP values. The outgroup is *F. concolor*. The NIV genotype is highlighted in blue and 15-ADON in green. FML: *Food Microbiology Laboratory*.



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2.3 Mycotoxin analysis

2.3.1 Occurrence of type B trichothecenes

Type B trichothecenes were found in 92% and 100% of oat samples from PR and RS, respectively. In PR, DON was the predominant mycotoxin and was detected in 44.2% of the samples; followed by NIV (28.6%), 3-ADON (18.8%) and 15-ADON (7.7%). In RS, NIV was detected in 44.7% of the samples and was the predominant mycotoxin; followed by DON (35%), 15-ADON (3.6%) and 3-ADON (14.8%).

Table 2 shows the levels of DON, 3-ADON, 15-ADON and NIV detected in the PR and RS regions. The mean contamination levels for mycotoxins in oat samples from PR were 45 µg/kg, 18.8 µg/kg, 7.7 µg/kg and 28.6 µg/kg for DON, 3-ADON, 15-ADON and NIV, respectively. Regarding the RS oat samples, the mean mycotoxin contamination levels were 35 µg/kg, 14.8 µg/kg, 3.6 µg/kg and 46.7 µg/kg for DON, 3-ADON, 15-ADON and NIV, respectively.

In the current study, a higher frequency and average concentration levels of NIV were found in RS; however, no significant difference was observed between RS and PR for the other evaluated mycotoxins ($p>0.05$).

The co-occurrence of trichothecenes was also observed. Eighty-four percent of the samples from the PR region simultaneously presented DON and NIV, whereas the co-occurrence of DON and NIV was observed in only 57.7% of the RS samples.

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Table 2. Occurrence of type B trichothecenes in oat grain samples from Rio Grande do Sul (RS) and Paraná (PR), Brazil.

Region	NIV			DON			15-ADON			3-ADON		
	Concentration (µg/kg)			Concentration (µg/kg)			Concentration (µg/kg)			Concentration (µg/kg)		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
PR	28.6	330.3	ND-820	45	540.1	ND-1,620	7.7	349.7	ND-723.3	18.8	648.6	ND-2,546.7
RS	46.7	778.3	ND-7,716.7	35	503.2	ND-1,610	3.6	157.8	ND-420	14.8	491.7	ND-3,333.3

NIV=nivalenol; DON=deoxynivalenol; 15-ADON=15-acetildeoxynivalenol; 3-ADON=3-acetildeoxynivalenol; ND=Not Detected.

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3 Discussion

The current study found a high diversity of fungi in Brazilian oat grains; including potentially toxigenic fungi. The occurrence of the FSAMSC and its related mycotoxins such as DON, 3-ADON, 15-ADON and NIV was the focus of the investigation. It is important to mention that there is a lack of research regarding mycotoxin contamination in Brazilian oat grains, despite high consumption by the population. Most of the previous studies were conducted in the Northern hemisphere and those have reported the presence of multiple *Fusarium* mycotoxins in oat grains [36,37].

A study conducted on Swiss oat samples from the 2013 to 2015 harvests reported the occurrence of nine different *Fusarium* species and a 97% frequency of *Fusarium* infection in the analyzed samples; similar to the frequency determined in this study (93.8% and 85.5% for samples from RS and PR, respectively). The same study pointed out that *F. poae* was the most predominant species in all three harvest years (2013, 2014 and 2015) with 55%, 57% and 87% isolation amongst *Fusarium* species [6].

In the current study, most of *Fusarium* isolates belonged to the FSAMSC and were characterized as *F. graminearum*, *F. meridionale* and *F. poae*. The latter was frequently isolated from RS samples, in contrast to PR samples; where *F. meridionale* was highly detected. Studies have highlighted *F. poae* as a frequent species found in oat samples [6,38]. These results suggest that different geographic origins, soil type, environmental and harvest conditions could lead to a distinct predominant species, and might influence the mycotoxin content of the grains [39].

In both studied regions, the NIV genotype was predominant. In RS, it was associated with the high frequency of *F. poae*, and with the samples mostly contaminated with NIV mycotoxin. The high occurrence of this genotype in PR is associated to *F. meridionale* and *F. poae*. This knowledge is relevant to determine a more efficient prediction of the contamination by NIV;

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and may aid management strategies to control the occurrence of toxigenic fungi in barley from different geographic regions [40].

Mycotoxin analysis demonstrated that most of the samples were contaminated with type B trichothecenes. DON contamination was higher in samples from PR, while NIV was prevalent in RS. The presence of these mycotoxins conforms with the frequency of isolated fungi, as *F. poae* was the most isolated species in RS and *F. graminearum* in PR. It has been reported that the incidence of *F. poae* increases when the climatic conditions do not favor the proliferation of *F. graminearum* s.s., the dominant pathogen involved in FHB [41,42].

In Brazil, previous studies revealed a high frequency of the FSAMSC in wheat, barley and rice, leading to the high occurrence of DON in grains. Despite this knowledge, information correlating mycotoxin contamination to the predominant species in Brazilian oats is still scarce [43-50]. In Europe, the high occurrence of *F. poae* in cereals is responsible for NIV contamination [36,51]; while in Asia, NIV contamination is attributed to *F. asiaticum* [52,53]. In South America, NIV was found in wheat from Argentina and Brazil in lower frequency and levels than DON. Apparently, the higher frequency of DON is related to the higher risk of FHB epidemics caused by the predominance of no-till cropping and climate change in the subtropical environment of Southern of Brazil [44,54]. Furthermore, the analysis detected the presence of 3-ADON and 15-ADON in oats, with high levels of 3-ADON in samples from both regions studied. This result corroborates with the occurrence of the *F. graminearum* 3-ADON genotype. In Europe, the acetylated DON forms are reported in cereals like oats [51,55-57], maize and wheat [58].

In our study, 24% of the samples presented DON levels higher than the maximum limit established by Brazilian legislation (750 µg/kg). Despite the absence of legislation for NIV globally, this mycotoxin was present in high levels, mainly in samples from RS. The toxic effects of NIV are still inconclusive, although it has displayed immunotoxic and hematotoxic

effects; which can be critical to humans [12]. In the case of acetylated DON forms (15-ADON and 3-ADON), high levels of 3-ADON were observed in the grains from both regions. Information about its toxic effects in animals and humans are still scarce, but a study demonstrated that 15-ADON is more toxic than DON and 3-ADON [13].

Co-contamination of DON, 15-ADON, 3-ADON and NIV was observed in this study due to the presence of different fungi in the grains. To our knowledge, this is the first report demonstrating the co-occurrence of these mycotoxins in Brazilian oat grains and their correlation with associated *Fusarium* species. However, the co-occurrence of DON and NIV has already been reported in 86% of Brazilian wheat kernels analyzed [44], as well as in 29.6% of Brazilian barley samples [50]. The main concern about co-contamination is the possible interactions and potential synergistic effects that these mycotoxins may have on animal and human health. [59] reported that the toxic effects of DON are intensified when consumed with NIV in *in vitro* models.

Overall, studies about mycotoxin contamination in oat grains are relevant and necessary, to determine an efficient risk control plan; as the consumption of oats *in natura* plant-based beverages or cereal-based foods has been increasing, boosted mainly by its good nutritional features, such as a high protein and dietary fiber content [60].

Since the levels of mycotoxin contamination and the dominant species in cereals can change according to various environmental parameters, studies that elucidate the prevalence of toxigenic fungi in different geographic regions are vital for designing efficient control management strategies, aiding the producers in obtaining a safer product. The results of this study highlighted the importance of further research on the contamination of multiple *Fusarium* mycotoxins in oat grains and their by-products consumed in Brazil.

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4 Conclusion

This study has shown high recovery of *F. graminearum s.s.* and *F. poae* from Brazilian oat grains, as well as contamination by the mycotoxins DON, 3-ADON, 15-ADON and NIV. Samples were highly contaminated with type B trichothecenes; and that 24% of the samples contaminated with DON were at concentrations higher than permitted by Brazilian legislation. Co-occurrence of these mycotoxins in oat grains samples was also observed; indicating the importance for further studies on trichothecene contamination in oat by-products; as well as the toxic synergistic interactions of these mycotoxins to determine potential risks to animal and human health.

5 Materials and methods

5.1 Oat samples

One hundred oat grain samples were collected from the States of Paraná and Rio Grande do Sul (50 samples from each region), the two largest oat producing regions of Brazil. The grains were obtained after the cleaning and drying stages (up to maximum 60^{o} C) of the 2018 harvest. Sampling was performed using a grain auger at different points of the harvest batches. Each sample was homogenized and reduced to a sub-sample of 3 kg. Grains were transferred into polyethylene bags and kept at room temperature (for up to two days). The bags were then stored at -18^{o} C for mycotoxin analysis [45].

5.2 Water activity and identification of mycobiota

Water activity analysis of the grain samples was conducted using the equipment Aqua-Lab CX-2, Decagon Devices. Samples were analyzed in triplicate. The serial dilution technique was used for fungal isolation [61]. Aliquots of each dilution were plated onto Dichloran Rose Bengal

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Chloramphenicol (DRBC, Oxoid) agar and incubated for 5 days at 25° C, results were expressed in CFU/g.

Primary morphological characterization of the different genera was conducted according to [61], using Czapek Yeast Extract Agar (CYA) and Malt Extract Agar (MEA). Isolates belonging to the genus *Fusarium* were single-spored and plated onto Potato Dextrose Agar (PDA) and Carnation Leaf Agar (CLA) for further morphological characterization [62]. Isolates were stored in glycerol (60%) at -80° C.

5.3 Characterization of *Fusarium* isolates

The *Fusarium* isolates were initially identified as described above. Afterwards, 25 strains belonging to the *F. sambucinum* species complex (FGSC and *F. poae* isolates), were selected for sequencing and phylogenetic analyses. These isolates were selected in order to represent both regions studied (Paraná and Rio Grande do Sul). Sequencing reactions followed by phylogenetic analysis were performed on the *RPB2* locus [63-65].

Isolates were also characterized based on trichothecene genotyping (3-ADON, 15-ADON and NIV) by multiplex PCR, following the methodology proposed by [66].

5.4 DNA extraction, PCR and sequencing analyses of the *RPB2* gene

Fusarium cultures were grown on PDA for 5 days at 25° C and the DNA was extracted using Dneasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR reactions and primer sets were performed according to [64,67]. PCR products were purified with QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced using Applied Biosystems® 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) by the company Helixxa Bases for Life (Paulínia, SP, Brazil).

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The sequences were analyzed using Geneious v.6.0.6 (Biomatters, Auckland, New Zealand), and polymorphisms were confirmed by examining the chromatograms. For multiple alignment, nucleotide sequences were downloaded from National Centre for Biotechnology Information (NCBI) and aligned with the obtained *Fusarium* oat isolate sequences using the ClustalW plugin in Geneious v.6.0.6 (Supplementary Table 1).

5.5 Phylogenetic analysis

Maximum parsimony analysis was performed using PAUP 4.0b10 (Sinauer Associates, Sunderland, MA, USA) [68]. A heuristic search option with 1000 random additional sequences and tree-bisection-reconnection algorithm for branch-swapping were used to infer the most parsimonious tree. Gaps were treated as missing data. The Consistency Index (CI) and Retention Index (RI) were calculated to verify the homoplasy present. Clade stability was verified through bootstrap analysis with 1000 replicates (PAUP 4.0b10), Bayesian inference analysis was also performed using the MrBayes plugin in Geneious v.6.0.6, run with a 2,000,000-generation Monte Carlo Markov chain method with a burn-in of 10,000 trees. *Fusarium concolor* was used as outgroup. The phylogenies were visualized using FigTree v.1.4 (University of Edinburgh, Edinburgh, United Kingdom) [69].

5.6 Mycotoxin analysis

5.6.1 Mycotoxin extraction

Mycotoxin extraction was conducted using QuEChERS, according to the manufacturer's instructions. Initially, 300 g of oat grains were ground, and a subsequent sub-sample of 100 g was separated using a sieve (0.5 mm mesh 32, generating 0.5 mm particles) and homogenized. Then, 10 g of the ground sample was weighed and transferred into a 50 ml QuEChERS

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extraction tube, followed by 10 ml of ultrapure water and 10 ml of acetonitrile with 1.0% formic acid.

The sample was agitated vigorously for 1 minute and then centrifuged for 5 minutes at 5000 rpm. After, 3 mL of supernatant was transferred to a 15 ml RoC QuEChERS centrifuge tube, containing 900 mg MgCl and 150 mg PSA (Primary and Secondary Amine Exchange Material - KS0-8924). This was shaken vigorously for 30 seconds and centrifuged for 5 minutes at 3700 x g to separate the solid material. Finally, 1mL of the supernatant was transferred into a flask for the solution to be evaporated in a heated sand bath at 60^{o}C.

Subsequently, the residue was diluted with 1 mL of acetonitrile:water (70:30 v/v), mixed and filtered through a 0.22 µm PTFE hydrophobic membrane filter, and injected into a high-performance liquid chromatography with diode array detection.

5.6.2 Chromatography conditions

Chromatographic separation was performed through a high-performance liquid chromatograph (Shimadzu, Kyoto, Japan), Gemini C18 5.0 µm (250x4.6mm) chromatographic column, an auto-injector for injection handling of 20 µL and equipped with a diode-array detector SPD-M20A [70].

The mobile phase was composed of acetonitrile:water (70:30 v/v), with elution in isocratic mode and a flow rate of 0.5 mL min^{-1}, with a total analysis time of 15 minutes. The maximum absorption wavelength was 220 nm for 3-ADON, 15-ADON, DON and NIV.

Data was collected and processed using LC Solution-Shimadzu software. Limit of detection (LOD), limit of quantification (LOQ) and recovery were: 16.15, 2.5, 2.5 and 16.15 µg/Kg; 53.3, 8.3, 8.3 and 53.3 µg/Kg; 98%, 92%, 84% and 70% for DON, 3-ADON, 15-ADON and NIV, respectively.

5.7 Statistical analysis

Statistical analysis was performed using Statistix v.10 software. ANOVA and the Kruskal-Wallis test were chosen to assess the differences of *Fusarium* occurrence between the two studied regions, as well as the differences in mycotoxin levels between the two studied regions. Values of $p < 0.05$ were considered statistically significant.

Authorship contribution statement

Mariana Pinheiro: Investigation. Methodology. Formal analysis. Writing – original draft. **Caio H. T. Iwase:** Formal analysis. Writing – original draft. **Bruno G. Bertozzi:** Formal analysis. Investigation. Methodology. **Elem Caramês:** Formal analysis. Writing – review & editing. **Lorena Carnielli-Queiroz:** Investigation. Methodology. **Nádia C. Langaro:** Funding acquisition. Resources. **Eliana B. Furlong:** Funding acquisition. Resources. **Benedito Correa:** Conceptualization. Funding acquisition**. Liliana O. Rocha:** Conceptualization. Funding acquisition. Resources. Project administration. Supervision. Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Kaur, S.; Bhardwaj, R.D.; Kapoor, R.; Grewal., S.K. Biochemical characterization of oat (*Avena sativa* L.) genotypes with high nutritional potential. *LWT* **2019**, *110*, 32-39.

2. United States Department of Agriculture (USDA). Grain: World markets and trade. Foreign Agricultural Service/USDA, Global Market Analysis. https://apps.fas.usda.gov/psdonline/circulars/grain.pdf (accessed 01 March 2021).

3. Liu, L.; Ma, M.; Liu, Z.; Zhang, L.; Zhou, J. Community structure of fungal pathogens causing spikelet rot disease of naked oat from different ecological regions of China. *Sci Rep* **2021**, *11*, 1243.

4. Tamburic-Ilincic, L. *Fusarium* species and mycotoxins associated with oat in southwestern Ontario, Canada. *Can J Plant Sci* **2010**, *90*, 211-216.

5. Hautsalo, J.; Jalli, M.; Manninen, O.; Veteläinen, M. Evaluation of resistance to *Fusarium graminearum* in oats. *Euphytica* **2018**, *214*, 139.

6. Schöneberg, T.; Jenny, E.; Wettstein, F.E.; Bucheli, T.D.; Mascher, F.; Bertossa, M.; Musa, T.; Seifert, K.; Gräfenhan, T.; Keller, B.; Vogelgsang, S. Occurrence of *Fusarium* species and mycotoxins in Swiss oats – Impact of cropping factors. *Eur J Agron* **2018**, *92*, 123-132.

7. Ghimire, B.; Sapkota, S.; Bahri, B.A.; Martinez-Espinoza, A.D.; Buck, J.W.; Mergoum, M. *Fusarium* Head Blight and rust diseases in soft red winter wheat in the Southeast United States: State of the art, challenges and future perspective for breeding. *Front Plant Sci* **2020**, *11*, 1080.

8. Mylona, K.; Magan, N. *Fusarium langsethiae*: Storage environment influences dry matter losses and T2 and HT-2 toxin contamination of oats. *J Stored Prod Res* **2011**, *47*, 321-327.

9. O'Donnel, K.; Rooney, A.P.; Proctor, R.H.; Brown, D.W.; McCormick, S.P.; …; Geiser, D.M. Phylogenetic analyses of *RPB1* and *RPB2* support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. *Fungal Genet Biol* **2013**, *52*, 20-31.

10. McCormick, S.P.; Stanley, A.M.; Stover, N.A.; Alexander, N.J. Trichothecenes: from simple to complex mycotoxins. *Toxins* **2011**, *3*, 802-814.

11. Pereira, C.S.; Cunha, S.C.; Fernandes, J.O. Prevalent mycotoxins in animal feed: Occurrence and analytical methods. *Toxins* **2019**, *11*, 290.

12. European Food Safety Authority (EFSA). Scientific opinion on risks for animal and public health related to the presence of nivalenol in food and feed. *EFSA Journal* **2013**, *11*, 3262.

13. Pinton, P.; Tsybulskyy, D.; Lucioli, J.; Laffitte, J.; Callu, P.; Lyazhri, F.; Grosjean, F.; Bracarense, A.P.; Kolf-Clauw, M.; Oswald, I.P. Toxicity of deoxynivalenol and its acetylated derivatives on the intestine: differential effects on morphology, barrier function, tight junction proteins, and mitogen-activated protein kinases. *Toxicol Sci* **2012**, *130*, 180-190.

14. World Health Organization (WHO). Safety evaluation of certain contaminants in food. Deoxynivalenol. **2011**.

15. Agriopoulou. S.; Stamatelopoulou, E.; Varzakas, T. Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods. *Foods* **2020**, *9*, 137.

16. World Health Organization (WHO). (2000). Evaluation of certain food additives and contaminants. Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives. **2000**.

17. Tekauz, A.; Mitchell Fetch, J.W.; Rossnagel, B.G.; Savard, M.E. Progress in assessing the impact of *Fusarium* head blight on oat in Western Canada and screening of avena germplasm for resistance. *Cereal Res Commun* **2008**, *36*, 49-56.

18. Yli-Mattila, T. Ecology and evolution of toxigenic *Fusarium* species in cereals in Northern Europe and Asia. *J Plant Pathol* **2010**, *92*, 7-18.

19. Schöneberg, T.; Kibler, K.; Wettstein, F.E.; Bucheli, T.D.; Forrer, H.R.; Musa, T.; Mascher, F.; Bertossa, M.; Keller, B.; Vogelgsang, S. Influence of temperature, humidity duration and growth stage on the infection and mycotoxin production by *Fusarium langsethiae* and *Fusarium poae* in oats. *Plant Pathol* **2019**, *68*, 173-184.

20. Kokkonen, M.; Jestoi, M.; Laitila, A. Mycotoxin production of *Fusarium langsethiae* and *Fusarium sporotrichioides* on cereal-based substrates. *Mycotoxin Res* **2012**, *28*, 25-35.

21. Stenglein, S.A.; Dinolfo, M.I.; Barros, G.; Bongiorno, F.; Chulze, S.N.; Moreno, M.V. *Fusarium poae* pathogenicity and mycotoxin accumulation on selected wheat and barley genotypes at a single location in Argentina. *Plant Dis* **2014**, *98*, 1733-1738.

22. Munkvold, G.P. *Fusarium* species and their associated mycotoxins. In *Mycotoxigenic Fungi: Methods and Protocols*; Moretti, A.; Susca, A., Eds.; Human Press, 2017, pp. 51-106.

23. Langseth, W.; Rundberget, T. The occurrence of HT-2 toxin and other trichothecenes in Norwegian cereals. *Mycopathologia* **1999**, *147*, 157-165.

24. Hietaniemi, V.; Kontturi, M.; Rämö, S.; Eurola, M.; Kangas, A.; Niskanen, M.; Saastamoinen, M. Contents of trichothecenes in oats during official variety, organic cultivation and nitrogen fertilization trials in Finland. *Agr Food Sci* **2004**, *13*, 54-67.

25. Gottschalk, C.; Barthel, J.; Engelhardt, G.; Bauer, J.; Meyes, K. Occurrence of type A trichothecenes in conventionally and organically produced oats and oat products. *Mol Nutr Food Res* **2007**, *51*, 1547-1553.

26. Nielsen, L.K.; Jensen, J.D.; Nielsen, G.C.; Jensen, J.E.; Spliid, N.H.; Thomsen, I.K.; Justesen, A.F.; Collinge, D.B.; Jørgensen, L.N. *Fusarium* head blight of cereals in Denmark: species complex and related mycotoxins. *Phytopathol* **2011**, *101*, 960-969.

27. Polišenská, I.; Jirsa, O.; Vaculová, K.; Pospíchalová, M.; Wawroszova, S.; Frydrych, J. *Fusarium* mycotoxins in two hulles oat and barley cultivars used for food purposes. *Foods* **2020**, *9*, 1037.

28. Rupollo, G.; Gutkoski, L.C.; Martins, I.R.; Elias, M.C. Efeito da umidade e do período de armazenamento hermético na contaminação natural por fungos e a produção de micotoxinas em grãos de aveia. *Cienc Agrotec* **2006**, *30*, 118-125.

29. Marini, L.J.; Gutkoski, L.C.; Elias, M.C.; Santin, J.A. Qualidade de grãos de aveia sob secagem intermitente em altas temperaturas. *Cienc Rural* **2007**, *37*, 1268-1273.

30. Sacchi, C.; González, H.H.L.; Broggi, L.E.; Pacin, A.; Resnik, S.L.; Cano, G.; Taglieri, D. Fungal contamination and mycotoxin natural occurrence in oats for race horses feeding in Argentina. *Anim Feed Sci Tech* **2009**, *152*, 330-335.

31. Diaz, G.; Perilla, N.; Rojas, Y. Occurrence of aflatoxins in selected Colombian foods. *Mycotoxin Res* **2001**, *17*, 15-20.

32. Mallmann, C.A.; Santurio, J.M.; Almeida, C.A.A.; Dilkin, P. Fumonisin B1 levels in cereals and feeds from Southern Brazil. *Arq Inst Biol* **2001**, *68*, 41-45.

33. Ortiz, J.; Camp, J.V.; Mestdagh, F.; Donoso, S.; Meulenaer, B. Mycotoxin co-occurrence in rice, oat flakes and wheat noodles used as staple foods in Ecuador. *Food Addit Contam A* **2013**, *30*, 2165-2176.

34. Ortiz, J.; Jacxsens, L.; Astudillo, G.; Ballesteros, A.; Donoso, S.; Huybregts, L.; Meulenaer, B. Multiple mycotoxin exposure of infants and young children via breastfeeding and complementary/weaning foods consumption in Ecuadorian highlands. *Food Chem Toxicol* **2018**, *118*, 541-548.

35. Graichen, F.A.S.; Martinelli, J.A.; Wesp, C.L.; Federizzi, L.C.; Chaves, M.S. Epidemiological and histological components of crown rust resistance in oat genotypes. *Eur J Plant Pathol* **2011**, *131*, 497-510.

36. Fredlund, E.; Gidlund, A.; Sulyok, M.; Börjesson, T.; Krska, R.; Olsen, M.; Lindblad, M. Deoxynivalenol and other selected *Fusarium* toxins in Swedish oats — Occurrence and correlation to specific *Fusarium* species. *Int J Food Microbiol* **2013**, *167*, 276-283.

37. Vidal, A.; Marín, S.; Ramos, A.J.; Cano-Sancho, G.; Sanchis, V. Determination of aflatoxins, deoxynivalenol, ochratoxin A and zearalenone in wheat and oat-based bran supplements sold in the Spanish market. *Food Chem Toxicol* **2013**, *53*, 133-138.

38. Hofgaard, I.S.; Aamot, H.U.; Torp, T.; Jestoi, M.; Lattanzio, V.M.T.; Klemsdal, S.S.; Waalwijk, C.; Van Der Lee, T.; Brodal, G. Associations between *Fusarium* species and mycotoxins in oats and spring wheat from farmers fields in Norway over a six-year period. *World Mycotoxin J* **2016**, *9*, 365–378.

39. Schöneberg, T.; Martin, C.; Wettstein, F. E.; Bucheli, T. D.; Mascher, F.; Bertossa, M.; Musa, T.; Keller, B.; Vogelgsang, S. *Fusarium* and mycotoxin spectra in Swiss barley are affected by various cropping techniques. *Food Addit Contam A* **2016**, *33*, 1608–1619.

40. Zingales, V.; Fernández-Franzón, M.; Ruiz, M. J. (2021). Occurrence, mitigation and in vitro cytotoxicity of nivalenol, a type B trichothecene mycotoxin – Updates from the last decade (2010–2020). *Food Chem Toxicol* **2021**, *152*, 112182.

41. Covarelli, L.; Beccari, G.; Prodi, A.; Generotti, S.; Etruschi, F.; Juan, C.; Ferrer, E.; Manes, J. *Fusarium* species, chemotype characterization and trichothecene contamination of durum and soft wheat in an area of Central Italy. *J Sci Food Agr* **2015**, *95*, 540-551.

42. Sumíková, T.; Chrpová, J.; Džuman, Z.; Salava, J.; Štěrbová, L.; Palicová, J.; Slavíková, P.; Stránská-Zachariášová, M.; Hajšlová, J. Mycotoxins content and its association with changing patterns of *Fusarium* pathogens in wheat in the Czech Republic. *World Mycotoxin J* **2017**, *10*, 143–151.

43. Almeida, M.I.; Almeida, N.G.; Carvalho, K.L.; Gonçalves, G.A.A.; Silva, C.N.; Santos, E.A.; Garcia, J.C.; Vargas, E.A. Co-occurrence of aflatoxins B1, B2, G1 and G2, ochratoxin A, zearalenone, deoxynivalenol, and citreoviridin in rice in Brazil. *Food Addit Contam A* **2012**, *29*, 694-703.

44. Del Ponte, E.M.; Garda-Buffon, J.; Badiale-Furlong, E. Deoxynivalenol and nivalenol in commercial wheat grain related to *Fusarium* head blight epidemics in southern Brazil. *Food Chem* **2012**, *132*, 1087-1091.

45. Piacentini, K.C.; Savi, G.D.; Pereira, M.E.; Scussel, V.M. Fungi and the natural occurrence of deoxynivalenol and fumonisins in malting barley (*Hordeum vulgare* L.). *Food Chem* **2015**, *187*, 204-209.

46. Calori-Domingues, M.A.; Bernardi, C.M.; Nardin, M.S.; Souza, G.V.; Santos, F.G.R.; Stein, M.A.; Gloria, E.M.; Dias, C.T.S.; Camargo, A.C. Co-occurrence and distribution of deoxynivalenol, nivalenol and zearalenone in wheat from Brazil. *Food Addit Contam B* **2016**, *9*, 142-151.

47. Tralamazza, S.M.; Bemvenuti, R.H.; Zorzete, P.; Garcia, F.S.; Corrêa, B. Fungal diversity and natural occurrence of deoxynivalenol and zearalenone in freshly harvested wheat grains from Brazil. *Food Chem* **2016**, *196*, 445-450.

48. Piacentini, K.C.; Rocha, L.O.; Savi, G.D.; Carnielli-Queiroz, L.; Almeida, F.G.; Minella, E.; Corrêa, B. Occurrence of deoxynivalenol and zearalenone in brewing barley grains from Brazil. *Mycotoxin Res* **2018**, *34*, 173-178.

49. Savi, G.D.; Piacentini, K.C.; Rocha, L.O.; Carnielli-Queiroz, L.; Furtado, B.G.; Scussel, R.; Zanoni, E.T.; Machado-de-Ávila, R.A.; Corrêa, B.; Angioletto, E. Incidence of toxigenic fungi and zearalenone in rice grains from Brazil. *Int J Food Microbiol* **2018**, *270*, 5-13.

50. Iwase, C.H.T.; Piacentini, K.C.; Giomo, P.P.; Čumová, M.; Wawroszová, S.; Běláková, S.; Minelli, E.; Rocha, L.O. Characterization of the *Fusarium sambucinum* species complex and detection of multiple mycotoxins in Brazilian barley samples. *Food Res Int* **200**, *136*, 109336.

51. Scudamore, K.A.; Bailie, H.; Patel, S.; Edwards, S.G. Occurrence and fate of *Fusarium* mycotoxins during commercial processing of oats in the UK. *Food Addit Contam* **2007**, *24*, 1374-1385.

52. Ndoye, M.; Zhang, J.B.; Wang, J.H.; Gong, A.D.; Li, H.P.; Qu, B.; Li, S.J.; Liao, Y.C. Nivalenol and 15-acetyldeoxynivalenol chemotypes of *Fusarium graminearum* clade species are prevalent on maize throughout China. *J Phytopathol* **2012**, *160*, 519-524.

53. Jang, J.Y.; Baek, S.G.; Choi, J.H.; Kim, S.; Kim, J.; Kim, D.W.; Yun, S.H.; Lee, T. Characterization of nivalenol-producing *Fusarium asiaticum* that causes cereal head blight in Korea. *Plant Pathol J* **2019**, *35*, 543-552.

54. Fernandez-Pinto, V.E.; Terminiello, L.A.; Basilico, J.C.; Ritieni, A. Natural occurrence of nivalenol and mycotoxigenic potential of *Fusarium graminearum* strains in wheat affected by Head Blight in Argentina. *Braz J Microbiol* **2008**, *39*, 157-162.

55. Schollenberger, M.; Müller, H.M.; Rüfle, M.; Suchy, S.; Planck, S.; Drochner, W. Survey of *Fusarium* toxins in foodstuffs of plant origin marketed in Germany. *Int J Food Microbiol* **2005**, *97*, 317-326.

56. Edwards, S.G. *Fusarium* mycotoxin content of UK organic and conventional oats. *Food Addit Contam A* **2009**, *26*, 1063-1069.

57. Hietaniemi, V.; Rämö, S.; Yli-Matila, T.; Jestoi, M.; Peltonen, S.; Kartio, M.; Sieviläinen, E.; Koivisto, T.; Parikka, P. Update survey of *Fusarium* species and toxins in Finnish cereal grains. *Food Addit Contam A* **2016**, *33*, 831-848.

58. Yan, P.; Liu, Z.; Liu, S.; Yao, L.; Liu, Y.; Wu, Y.; Gong, Z. Natural occurrence of deoxynivalenol and its acetylated derivatives in Chinese maize and wheat collected in 2017. *Toxins* **2020**, *12*, 200.

59. Cheat, S.; Gerez, J.R.; Cognié, J.; Alassane-Kpembi, I.; Bracarense, A.P.F.L.; Raymond-Letron, I.; Oswald, I.P.; Kolf-Clauw, M. Nivalenol has a greater impact than deoxynivalenol on pig jejunum mucosa *in vitro* on explants and *in vivo* on intestinal loops. *Toxins* **2015**, *7*, 1945-1961.

60. Spaen, J.; Silva, J. V. C. Oat proteins: Review of extraction methods and techno-functionality for liquid and semi-solid applications. *LWT* **2021**, *147*, 111478.

61. Pitt, J.I.; Hocking, A.D. *Fungi and food spoilage*. Springer, Boston, 2009.

62. Leslie, J.F.; Summerell, B.A. *The Fusarium laboratory manual*. Wiley-Blackwell, Iowa, 2006.

63. O'Donnell, K.; Kistler, H.C.; Cigelnik, E.; Ploetz, R.C. Multiple evolutionary origins the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *PNAS* **1998**, *95*, 2044-2049.

64. O'Donnell, K.; Sutton, D.A.; Rinaldi, M.G.; Sarver, B.A.J.; Balajee, S.A.; Schroers, H.J.; …; Geiser, D.M. Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. *J Clin Microbiol* **2010**, *48*, 3708–3718.

65. Laurence, M.H.; Summerell, B.A.; Liew, E.C.Y. *Fusarium oxysporum* f. sp. *canariensis*: evidence for horizontal gene transfer of putative pathogenicity genes. *Plant Pathol* **2015**, *64*, 1068-1075.

66. Ward, T.J.; Bielawski, J.P.; Kistler, H.C.; Sullivan, E.; O'Donnell, K. Ancestral polymorphism and adaptative evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. *PNAS* **2002**, *99*, 9278-9283.

67. O'Donnell, K.; Sarver, B.A.J.; Brandt, M.; Chang, D.C.; Noble-Wang, J.; Park, B.J.; …; Ward, T.J. Phylogenetic diversity and microsphere array-based genotyping of human pathogenic fusaria, including isolates from the multistate contact lens-associated U.S. keratitis outbreaks of 2005 and 2006. *J Clin Microbiol* **2007**, *45*, 2235–2248.

68. Swofford, D.L. PAUP* phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, 2002.

69. Rambaut, A. FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, 2010.

70. Arraché, E.R.S.; Fontes, M.R.V.; Buffon, J.G.; Badiale-Furlong, E. Trichothecenes in wheat: Methodology, occurrence and human exposure risk. *J Cereal Sci* **2018**, *82*, 129-137.