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Trichothecenes and Mycoflora in Wheat Harvested in Nine Locations in Buenos Aires Province, Argentina

H. H. L. González · G. A. Moltó · A. Pacin · S. L. Resnik · M. J. Zelaya · M. Masana · E. J. Martínez

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Abstract A total of 120 freshly harvested wheat samples from the 2004 season in nine locations from Northern Buenos Aires Province, Argentina, were analysed for trichothecene natural occurrence and associated mycoflora, and for determining the influence of commonly used fungicide field treatment and the cultivar type on trichothecene contamination. The trichothecenes T-2 tetraol, T-2 triol, HT-2 and T-2 toxin (HT-2, T-2), diacetoxyscirpenol (DAS), nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) were analysed by gas chromatography and electron capture detection. Detection limits ranged from 4 to 20 μg/kg. The isolation frequencies of species were calculated. *Alternaria alternata*, *Fusarium graminearum, Fusarium poae* and *Fusarium semitectum* were the predominant fungal species identified as endogenous mycoflora. The type of cultivar and the fungicide field treatment did not affect significantly the trichothecene contamination. The trichothecenes type A detected were

H. H. L. González
Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

H. H. L. González
Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad de Buenos Aires, Buenos Aires, Argentina

H. H. L. González (✉)
Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, C1428EHA Buenos Aires, Argentina
e-mail: glucas@speedy.com.ar

G. A. Moltó · S. L. Resnik
Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

G. A. Moltó
Facultad de Bromatología, Universidad Nacional de Entre Ríos, Gualeguaychú, Argentina

A. Pacin · M. J. Zelaya
Fundación de Investigaciones Científicas Teresa Benedicta de la Cruz, Luján, Argentina

A. Pacin · S. L. Resnik
Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Buenos Aires, Argentina

A. Pacin
Universidad Nacional de Luján, Lujan, Argentina

M. Masana
Instituto Tecnología de Alimentos, INTA Castelar, Castelar, Argentina

E. J. Martínez
Instituto de Cálculo, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

HT-2 and T-2 triol toxins and the type B were DON, NIV and 3-ADON. Based on 120 samples the incidences were 21.7% for 3-ADON, 22.5% for HT-2, 27.5% for T-2 triol and 85% for DON. NIV was confirmed in one sample. Mean levels of trichothecene positive samples were between 7 and 2788 μg/kg.

Keywords *Alternaria* · Deoxynivalenol · *Fusarium* · Mycoflora · Trichothecenes · Wheat

Abbreviations

3-ADON	3-Acetyldeoxynivalenol
15-ADON	15-Acetyldeoxynivalenol
DAS	4,15 Diacetoxyscirpenol
DON	Deoxynivalenol
NIV	Nivalenol
HT-2	HT-2 toxin
T-2	T-2 toxin
T-2 4OH	T-2 tetraol toxin
T-2 3OH	T-2 triol toxin

Introduction

Fusarium fungi may be a serious problem in the complete cereal food and feed chain because they contaminate the grain with mycotoxins, which cause serious illness and immuno-repression in humans and animals. Wheat infection by *Fusarium* causes yield losses and can result in DON contamination that is the trichothecene that more frequently contaminates this cereal [1].

Much evidence is available elsewhere on wheat mycoflora contamination as well as the *Fusarium* species associated. In the USA and Canada the predominant *Fusarium* species was *Fusarium graminearum* [2] while in Europe the most common isolates were *F. graminearum*, *Fusarium culmorum*, *Fusarium avenaceum* and *Fusarium poae* and were also associated with trichothecenes occurrence in wheat samples [3].

Bread wheat (*Triticum aestivum* L.) is the main cereal used for human consumption in Argentina [4, 5]. Since 1985 studies performed in Argentinian wheat and by-products showed the occurrence of DON [6–11].

Information is available regarding the mycoflora of Argentinian wheat, its toxigenic capacity and on their relationship with DON natural occurrence. In Argentina there are climatic differences between agroecological regions that could influence the trichothecene contamination. On the other hand during the last 10 years genetic germoplasm from China and Europe were introduced for breeding purposes and this could determine the type of *F. graminearum* chemotype present.

Since 1990 the presence in Argentina of 3-ADON and 15-ADON producers has been shown by Faifer et al. [12]. These findings were confirmed in a study done on the chemotypes found in Argentinian wheat [13] where presence of DON and acetyl derivates (3-ADON and 15-ADON) chemotype, NIV and acetyl derivates chemotype and DON-NIV and acetyl derivates chemotype was observed, in agreement with Lori et al. [14]. In the region of Santa Fe Province predominated the DON and acetyl derivates chemotype, in Buenos Aires Province occurred the DON-NIV and acetyl derivates chemotype and NIV was detected in subregions II N and II S.

Alvarez et al. [15] reported that all the tested strains (250) were of lineage 7 and DON and acetyl derivates chemotype. However in the last 10 years, only one survey was done on trichothecene type A and B co-occurrence. For this reason the aims of this study were to identify the mycoflora present on commercial bread wheat cultivars freshly harvested in the major Argentinian wheat production area, to study the trichothecene type A and B natural occurrence and to analyse if any influence of fungicide treatment and cultivar type was observed on trichothecene and fungal contamination.

Materials and Methods

Wheat Samples

A total of 120 freshly harvested bread wheat samples (≥3 kg) were collected during the 2003/2004 harvest season (early December 2003 to end of January 2004) in experimental fields belonging to INTA (Instituto Nacional de Tecnología Agropecuaria) from yearly field trials carried out to study the yield performance of commercial cultivars. The experimental fields were at nine locations in the major wheat production area corresponding to region II in Northern Buenos Aires Province (Fig. 1). This area

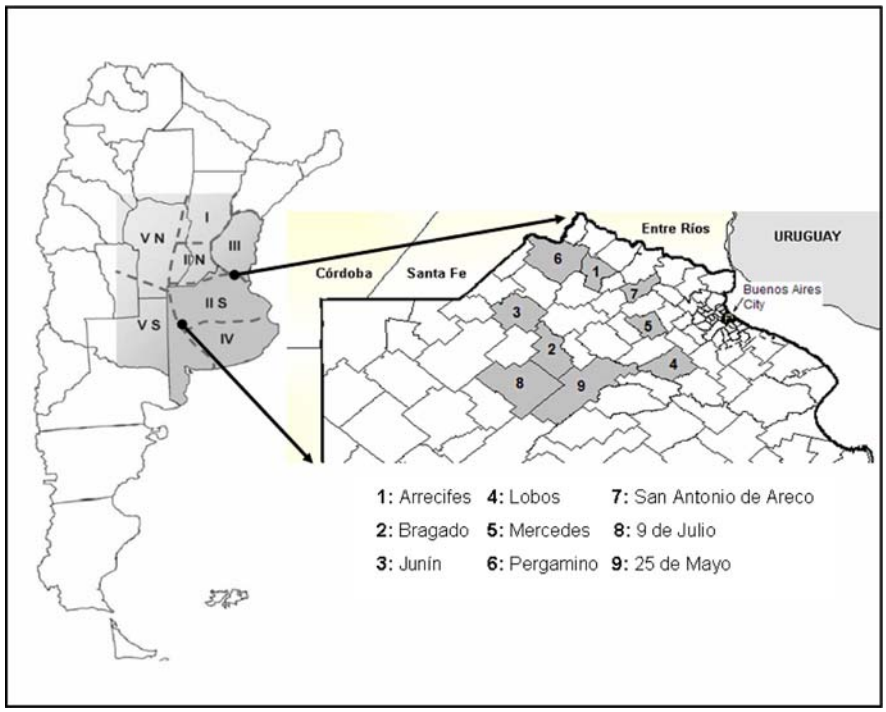

Fig. 1 Geographical distribution of the locations tested in 2004 in the North of Buenos Aires Province, Argentina

has temperate-humid climate. The locations were: Arrecifes (12 samples), Bragado (14), Junín (14), San Antonio de Areco (14), Lobos (7), Mercedes (15), 9 de Julio (21), 25 de Mayo (9) and Pergamino (14). The wheat cultivars tested were ACA 303, Baguette 10, Baguette 13, Churrinche, Gaucho, Molinero and Tijereta, having been selected because they were the most cultivated in region II. For each location, the plots of each wheat cultivar were either treated or not with fungicide applied in Zadoks 39 stage (flag leaf ligule/collar just visible). The fungicide was Sphere (trifloxystrobin 18.75% + cyproconazole 8%).

Randomly selected subsamples of 500 g each were submitted for mycological and mycotoxin analysis.

Isolation and Identification of Fungi

For isolation of the mycoflora in whole grains, subsamples of wheat kernels from each sample were surface-disinfected in a commercial 5% aqueous solution of sodium hypochlorite for 1 min, rinsed twice with sterile distilled water and dried in a sterile laminar flow cabinet. One hundred kernels per subsample were placed, 20 kernels per plate, on dichloran chloramphenicol peptone agar (DCPA). This selective medium was chosen because it allows growth of both *Fusarium* and dematiaceous fungi [16]. The plates were incubated in the dark at 28°C for 4–7 days and the resulting fungal colonies subcultured onto PDA (Potato-Dextrose Agar, Merck No 10130). Where several different fungi were isolated from a single kernel, all were recorded.

Isolates of fungi were transferred from PDA plates to their respective identification media according to the following authorities: *Fusarium* spp. according to Nelson et al. [17]; Dematiaceous fungi, *Aspergillus* spp., *Penicillium* spp. and other fungi according to Pitt et al. [18], Samson et al. [19] and Klich [20].

The isolation frequency (Fr) and the relative density (RD) of genera and species were calculated according to González et al. [7]:

$$\mathrm{Fr}(\%) = \left(\frac{\mathrm{ns}}{N}\right) \times 100 \quad \mathrm{RD}(\%) = \left(\frac{\mathrm{ni}}{\mathrm{Ni}}\right) \times 100,$$

where, ns = number of samples where a genus or species of fungi occurred; N = total number of samples; ni = number of isolates of a genus or species; Ni = total number of fungal isolates obtained.

Some of the cultures have been deposited in the BAFC Culture Collection from the Facultad de Ciencias Exactas y Naturales of the University of Buenos Aires in the Biodiversity and Experimental Biology Department.

Trichothecenes Analysis

Reagents

Organic solvents were of HPLC grade from Merck (Darmstadt, Germany). HPLC quality water was prepared with a Waters Milli-Q system (Waters Associated, Milford, MA, USA). 4-*N*,*N*-dimethylaminopyridine (DMAP) and Standards of the trichothecenes type A :T-2, HT-2, T-2 4OH, T-2 3OH and DAS and type B: DON, 3-ADON, 15-ADON and NIV were from Sigma Chemical Company (St. Louis, Mo, United States of America). Deepoxy-deoxynivalenol (ε-DON) was from Biopure (BRM 502033), Heptafluorbutyric anhydride (HFBA) were purchased from Interchemistry (Avocado® Research Chemical Ltd., 10744), and 2-amino-5-chlorobenzo-phenone (ACBP) was from Sigma (A-4632).

Extraction

Trichothecenes extraction of the ground samples was performed as described for DON by Trucksess et al. [21] with slight modifications according to Samar et al. [22]. Subsamples (25 g) were extracted with 100 ml of acetonitrile:water (84:16) by blending for 3 min at high speed with an Osterrizer blender.

Cleanup

Extracts (8 ml) were placed in an 8 × 15 mm culture tube and a 4 ml portion was passed through a Mycosep 227 column (Romer Labs. Inc., MO, USA), evaporated to dryness in a 40°C water bath under vacuum and stored at −18°C prior the analysis. The dried extract residues were derivatized with heptafluorobutyric anhydride (HFBA) as described by Croteau et al. [23] in the presence of 4-*N*,*N*-dimethylaminopyridine (4-DMAP), with ACBP and ε-DON as internal standard.

Derivatization Conditions

Briefly, the catalyst solution of DMAP was added to the dried extract (0.25 g equivalent weight, 168.5 ng ACBP and 25.3 ng ε-DON), vortexed and 50 µl of HFBA were added. The tube was placed in a sand bath at 60°C for 30 min. Excess of derivatizing agent were destroyed with 1.2 ml aqueous sodium bicarbonate solution (5% in distilled water) and 400 ml toluene were added. After centrifuging, the upper organic layer (480 ml) was transferred to an autosampler vial for GC analysis.

Apparatus

Gas chromatography with electron capture detection (GC-ECD) was performed on a Hewlett–Packard Model 5890 Series II equipped with an HP automatic liquid sampler, an HPG1512A controller, an HPG1513A injector module and an HP3398A GC-ChemStation. The GC separation was achieved with an HP-5 capillary column (30 m, 0.25 mm of i.d., 0.25 mm thickness of film). The temperature programme consisted of holding 1 min at 80°C, and then increasing the temperature at 30°C min^{-1} from 80 to 160°C, followed by an increase from 160 to 183°C at 1°C min^{-1} and then 183–280°C at 12°C min^{-1} (with 5-min hold). Column head pressure was 12 psi. Nitrogen flow was 1 ml min^{-1}. The temperature of the injector and the detector was 250 and 300°C, respectively. The injection volume was 2 µl. A standard curve was constructed to determine electron capture detector response to trichothecene standards with R^2 greater than 0.99 for all mycotoxins. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated at a signal-to-noise ratio of 3:1 and 5:1, respectively. The LOD and LOQ were for NIV: 7 and 11 µg/kg; for DON: 4 and 10 µg/kg; for T-2 4OH: 15 and 20 µg/kg; for ADON: 10 and 17 µg/kg; for T-2 3OH: 16 and 20 µg/kg; for DAS: 17 and 19 µg/kg and for HT-2 and T-2: 10 and 20 µg/kg.

Recovery from Grain Samples

Control ground wheat sample was extracted as described above and the sample was spiked with a mixture of trichothecenes in extraction solvent at different levels. Trichothecene recoveries with three replications spiked at level of 200 µg/kg were: for NIV: 78.6%, SD: 2.2; for DON: 108.2%, SD: 4.3; for T-2 4OH: 87.3%, SD: 3.7; for 3-ADON: 71.6%, SD: 2.3; for T-2 3OH: 98.2%, SD: 1.9; for HT-2: 83.0%,

SD: 1.5 and for T-2: 79.0%, SD: 4.3. For DAS, it was 78.0% at level 1,000 μg/kg, SD: 4.6. The spiked samples were cleaned up and derivatized as described above. Internal standards of ACPB and ε-DON were added.

Confirmatory Analysis

The dried sample extracts were resuspended in toluene:acetonitrile (95:5) and spotted on TLC plates (Merck art. 5553) against the corresponding standards. The plates were developed with freshly prepared solvent systems consisting of either chloroform:acetone:2-propanol (8:1:1) or toluene:ethyl acetate:formic acid (6:2:1). Type B trichothecenes (NIV, DON, 3-ADON and 15-ADON) exhibited blue fluorescence at 366 nm when the plates were treated with 20% ethanolic aluminium chloride solution and heated for 7 min at 120°C. Type A trichothecenes (DAS, T-2, HT-2, T-2 3OH and T-2 4OH) exhibit blue fluorescence at the same UV wavelength when the plates were sprayed with methanol:sulphuric acid:acetic acid (8:1:1) solution followed by heating at 130°C for 5 min. All samples with DON and·3-ADON contamination level higher than 25 μg/kg, and with T-2 3OH level higher than 50 μg/kg, were confirmed by TLC.

Statistical Analysis

Asymptotic tests were used to compare relative densities (RD) of fungal genera and species between locations [24].

The Fisher exact test [25] was applied to analyse possible differences in the isolation frequencies (Fr) of fungal genera between locations. To compare the DON contamination level between the different locations the median test was performed. If significant differences were found, multiple comparisons were done by using the Bonferroni correction in order to obtain a given global level [26]. According to the suggestion of the GEMS/Food EURO Second Workshop on Reliable Evaluation of Low Level Contamination of Food [27], the criteria adopted to estimate the trichothecene contamination when values less than the LOD are observed, were the following: When all observations are over the LOD then the true mean is calculated; when the proportion of observations less than LOD is lower than or equal to 60%, the mean is calculated replacing those observations by LOD/2 and when the proportion is over 60%, two estimates are informed: one obtained replacing those observations by 0 and the other replacing them by LOD. The statistical software Statistix 7.0 [28] was used.

Results and Discussion

Endogenous Wheat Mycoflora

In Table 1 the Fr and RD of fungi isolated in Arrecifes, Bragado, Junín, Pergamino and San Antonio de Areco in Buenos Aires Province are presented. Considering both isolation frequency and relative density, it can be seen that *Alternaria alternata* (4,348 isolates) was the most prevalent component of the internally seedborne mycoflora. Comparing the results of the present study with those observed in Entre Ríos Province (a warmer area than Buenos Aires Province) in 1999 [29], the *A. alternata* incidence in wheat was similar. In Buenos Aires province, with cool to temperate climate, the *A. alternata* incidence observed in the 1996 durum wheat harvest in region II N was also similar [8]. In 1993 during a serious *Fusarium* head blight outbreak in Argentina [7], the *A. alternata* incidence in the same region II and in region V N was lower than that determined in this study.

Members of the genus *Fusarium* were isolated predominantly as *F. graminearum* (RD: 18.3%), *F. poae* (RD: 1.9%) and *Fusarium semitectum* (RD: 1.1%). The prevalence of *F. graminearum* in the bread wheat samples agree with those observed previously in the same region [7].

Other *Fusarium* spp. with minor incidence levels and also cited as trichothecene producers [1] were: *F. acuminatum*, *F. avenaceum*, *F. culmorum*, *F. equiseti* and *F. sambucinum*.

Aspergillus flavus and *Penicillium citrinum* were detected at very low levels of Fr and RD so the natural occurrence of mycotoxins produced by these species (aflatoxins, cyclopiazonic acid and citrinin) is not foreseen. For this reason in the 2004 harvest season their natural occurrence was not studied.

The mycoflora present and the fact that *F. graminearum* was the most prevalent internally seedborne

Table 1 Isolation frequency (Fr) and relative specific density (RD) of the endogenous mycoflora recovered from bread wheat harvested in Arrecifes, Bragado, Junín, Pergamino and San Antonio de Areco locations (Buenos Aires Province, Argentina) in 2004

	Arrecifes		Bragado		Junín		Pergamino		San Antonio de Areco	
Number of isolates	1,200		1,400		1,400		1,400		1,400	
Species	Fr	RD	Fr	RD	Fr	RD	Fr	RD	Fr	RD
Alternaria alternata	100.0	65.7	100.0	67.1	100.0	76.7	100.0	66.5	100.0	44.0
Aspergillus flavus	8.3	0.3	7.1	0.1	7.1	0.1	nd	nd	nd	nd
Arthrinium phaeospermum	25.0	0.8	7.1	0.1	nd	nd	nd	nd	14.3	0.5
Bipolaris sorokiniana	41.7	0.8	28.6	0.5	28.6	0.6	64.3	1.6	42.9	0.8
Cladosporium cladosporioides	66.7	1.7	71.4	1.9	28.6	0.9	64.3	1.6	85.7	1.9
Curvularia lunata	8.3	0.3	42.9	0.6	21.4	0.3	nd	nd	7.1	0.1
Epicoccum nigrum	100.0	11.3	92.9	7.5	78.6	4.9	92.9	10.6	92.9	8.4
Eurotium chevalieri	33.3	0.6	21.4	0.4	21.4	0.8	42.9	0.6	28.6	0.9
Fusarium acuminatum	nd	nd	nd	nd	nd	nd	nd	nd	7.1	0.1
Fusarium avenaceum	nd	nd	10.5	0.3	nd	nd	nd	nd	14.3	1.1
Fusarium culmorum	16.7	0.2	nd	nd	nd	nd	7.1	0.6	14.3	0.4
Fusarium equiseti	nd	nd	7.1	0.1	nd	nd	14.3	0.4	nd	nd
Fusarium graminearum	100.0	13.3	100.0	16.6	100.0	10.6	100.0	13.4	100.0	36.9
Fusarium poae	83.3	2.8	85.7	2.6	57.1	1.7	42.9	1.0	64.3	1.8
Fusarium sambucinum	25.0	0.9	7.1	0.1	nd	nd	nd	nd	7.1	0.3
Fusarium semitectum	25.0	0.5	21.4	0.5	50.0	1.6	57.1	1.9	28.6	0.9
Mucor racemosus	nd	nd	nd	nd	nd	nd	7.1	0.6	nd	nd
Nigrospora oryzae	33.3	0.7	42.9	1.5	21.4	0.9	35.7	1.3	42.9	2.1
Penicillium citrinum	25.0	0.4	14.3	0.3	21.4	0.8	7.1	0.1	nd	nd
Phoma spp.	nd	nd	nd	nd	7.1	7.1	0.4	0.1	nd	nd
Trichoderma harzianum	nd	nd	7.1	0.1	16.7	7.1	0.1	nd	nd	nd
Ulocladium spp.	nd	nd	nd	nd	7.1	0.1	nd	nd	nd	nd

nd: Not detected

Fusarium species in commercial Argentinian bread wheat hybrids harvested in 2004, agree with previous observations [7, 8, 11, 29] and differ from that found in other wheat-producing countries. The most frequently encountered *Fusarium* species in Europe were *F. graminearum* and *F. culmorum*, with the former more common in Southern (warmer) and the latter in Northern (colder) European areas [3]. In Northern European countries other toxigenic *Fusarium* species as *F. avenaceum, F. tricinctum* and *F. langsethiae* were also frequently isolated from kernels of wheat [30, 31]. Performing the asymptotic tests, highly significant differences ($P < 0.01$) were found when comparing the RDs between locations for *F. graminearum*, *A. alternata* and *F. semitectum.*

Comparing mycotoxigenic species Fr between locations using the Fisher exact test, significant statistical differences ($P < 0.0461$) were observed only for *F. poae*, between Bragado and Pergamino. This *Fusarium* species is a trichothecene producer (NIV, HT-2 and T-2) and was quoted as a dominant species in wheat in Europe, especially in warm weather conditions [32].

No statistical differences in *Fusarium* infection were detected in the comparison between wheat samples with and without trifloxystrobin 18.75% + cyproconazole 8% treatment applied in Zadoks 39 stage.

Trichothecene Analysis

The trichothecene contamination was analysed and the occurrence of DON, 3-ADON, NIV, HT-2 and T-2 3OH was confirmed. The descriptive statistics of

Table 2 Contamination by trichothecenes in freshly bread wheat samples collected in Buenos Aires Province, Argentina in 2004

Location	Statistics	Trichothecenes (μg/kg)				
		DON	NIV	3-ADON	H-T2	T-2 3OH
Arrecifes	Positive samples/samples	10/12	1/12	4/12	4/12	7/12
	Mean[a]	84.3	10.6[c] 4.2[d]	9.8	11.2	27.1
	Range of positive samples	7–754	0–50	14–22	13–41	21–121
	Mean[b]	96.4	50.0	17.0	21.5	42.9
	Median[b]	16.0	50.0	16.0	16.0	27.0
Bragado	Positive samples/samples	14/14	nd	nd	6/14	9/14
	Mean[a]	290.5	nd	nd	11.3	44.8
	Range of positive samples	69–562	nd	nd	15–21	21.0–123.0
	Mean[b]	290.5	nd	nd	17.7	68.5
	Median[b]	275.0	nd	nd	17.0	74.0
Junín	Positive samples/samples	7/14	nd	9/14	6/14	3/14
	Mean[a]	4.8	nd	16.9	10.1	12.9[c] 5.0[d]
	Range of positive samples	7–11	nd	14–43	10–32	21–28
	Mean[b]	7.6	nd	23.4	15.2	23.3
	Median[b]	7.0	nd	20.0	12.5	21.0
Lobos	Positive samples/samples	7/7	nd	nd	nd	nd
	Mean[a]	558.0	nd	nd	nd	nd
	Range of positive samples	83–1733	nd	nd	nd	nd
	Mean[b]	558.0	nd	nd	nd	nd
	Median[b]	421.0	nd	nd	nd	nd
Mercedes	Positive samples/samples	15/15	nd	nd	nd	nd
	Mean[a]	633.7	nd	nd	nd	nd
	Range of positive samples	118–1956	nd	nd	nd	nd
	Mean[b]	633.7	nd	nd	nd	nd
	Median[b]	520.0	nd	nd	nd	nd
Pergamino	Positive samples/samples	10/14	nd	8/14	9/14	5/14
	Mean[a]	13.7	nd	15.1	11.2	16.5
	Range of positive samples	7–66	nd	14–30	10–24	21–67
	Mean[b]	12.9	nd	22.6	14.7	34.0
	Median[b]	7.0	nd	23.5	14.0	28.0
San Antonio de Areco	Positive samples/samples	14/14	nd	5/14	2/14	9/14
	Mean[a]	1083.6	nd	8.9	10.5[c] 1.9[d]	27.2
	Range of positive samples	204–2438	nd	14–14	12–15	26–101
	Mean[b]	1083.6	nd	14.0	13.5	39.6
	Median[b]	974.0	nd	14.0	13.5	32.0
9 de Julio	Positive samples/samples	21/21	nd	nd	nd	nd
	Mean[a]	198.6	nd	nd	nd	nd
	Range of positive samples	52–777	nd	nd	nd	nd
	Mean[b]	198.6	nd	nd	nd	nd
	Median[b]	162.0	nd	nd	nd	nd

Table 2 continued

Location	Statistics	Trichothecenes (μg/kg)				
		DON	NIV	3-ADON	H-T2	T-2 3OH
25 de Mayo	Positive samples/samples	9/9	nd	nd	nd	nd
	Mean[a]	1271.1	nd	nd	nd	nd
	Range of positive samples	259–2788	nd	nd	nd	nd
	Mean[b]	1271.1	nd	nd	nd	nd
	Median[b]	749.0	nd	nd	nd	nd

nd: not detected

[a] Calculated according to the GEMS/Food-EURO Second Workshop [27]

[b] Over positive samples

[c] Mean calculated using LOD for not detected

[d] Mean calculated using 0 for not detected

trichothecenes are listed in Table 2. NIV was present in one sample in Arrecifes (50 μg/kg) and 15-ADON, T-2, T-2 4OH and DAS were never detected.

The 3-ADON was present in 21.7% of total wheat samples, HT-2 in 22.5% and T-2 3OH in 27.5%. In a recent study carried out in Germany in grains [33], similar values for mean contamination levels for HT-2 and 3-ADON were observed in stored wheat (over 41 wheat samples analysed).

In this study DON was the predominant trichothecene present in all locations (85% of total samples) with a minimum over positive samples of 7 μg/kg, a maximum of 2788 μg/kg and a media of 450.7 μg/kg. This incidence and the level content were similar to those observed by Schollenberger et al. in Germany [33]. In Table 2, it can be observed that the highest DON contamination levels corresponded to San Antonio de Areco, 25 de Mayo, Mercedes and Lobos.

A comparison between the DON contaminations at the different locations was also performed using the median test and the corresponding *P* values are presented in Table 3. It can be seen that almost all comparisons produce significant results.

No statistical differences in trichothecenes concentration were detected in the comparison between wheat cultivars samples collected at nine locations of Buenos Aires Province. The same situation was observed when the comparison of DON content between wheat samples with and without fungicide treatment was done in Zadoks 39

Table 3 *P* values for the comparison between deoxynivalenol contamination in wheat harvested at nine locations in Buenos Aires Province, Argentina during 2004

	1	2	3	4	5	6	7	8	9
1		0.00010	0.00001	0.00110	0.00010	0.00001	0.00010	0.00030	0.00010
2	0.00010		0.00100	0.32910	0.02330	0.00000	0.00250	0.48580	0.00240
3	0.00001	0.00100		0.00020	0.00140	0.00001	0.00100	0.00200	0.00040
4	0.00110	0.32910	0.00020		0.64710	0.00070	0.01910	0.19040	0.01170
5	0.00010	0.02330	0.00140	0.64710		0.00000	0.05810	0.00020	0.03500
6	0.00001	0.00000	0.00001	0.00070	0.00000		0.00000	0.00000	0.00001
7	0.00010	0.00250	0.00100	0.01910	0.05810	0.00000		0.00120	0.63920
8	0.00030	0.48580	0.00200	0.19040	0.00020	0.00000	0.00120		0.00200
9	0.00010	0.00240	0.00040	0.01170	0.03500	0.00001	0.63920	0.00200	

1: Arrecifes; 2: Bragado; 3: Junín; 4: Lobos; 5: Mercedes; 6: Pergamino; 7: San Antonio de Areco; 8: 9 de julio; 9: 25 de Mayo

$P < 0.0014$: differences are significant

$P < 0.0003$: differences are highly significant

stage, in spite of the fact that the highest DON contamination levels were observed in samples with fungicide treatment.

Conclusions

This study provides information on the mycoflora and mycotoxins present on commercial bread wheat cultivars freshly harvested in the main production area in Argentina during 2004. The fungal species associated with wheat in Argentina that should be of concern because of their toxigenic potential and prevalence include *F. graminearum, F. poae, F. semitectum*, *F. equiseti* and *A. alternata*.

The high incidence of *A. alternata* isolates in the endogenous mycoflora of the 2004 bread wheat harvest should be a matter of concern and the natural occurrence of *Alternaria* toxins in Argentinian wheat needs to be studied.

The prevalent trichothecene found was DON with contamination levels over positive samples ranging from 7 to 2,788 μg/kg. The highest contamination levels of DON were detected in San Antonio de Areco and 25 de Mayo.

The wheat cultivars and the commonly used fungicide field treatment did not influence significantly the DON level contamination. The relatively high level of natural DON contamination in region II could have been due to a high rain period that occurred in that area during the flowering stage (October–November).

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