Trichothecene genotypes, chemotypes and zearalenone production by Fusarium graminearum *species complex strains causing Fusarium head blight in Argentina during an epidemic and non-epidemic season*

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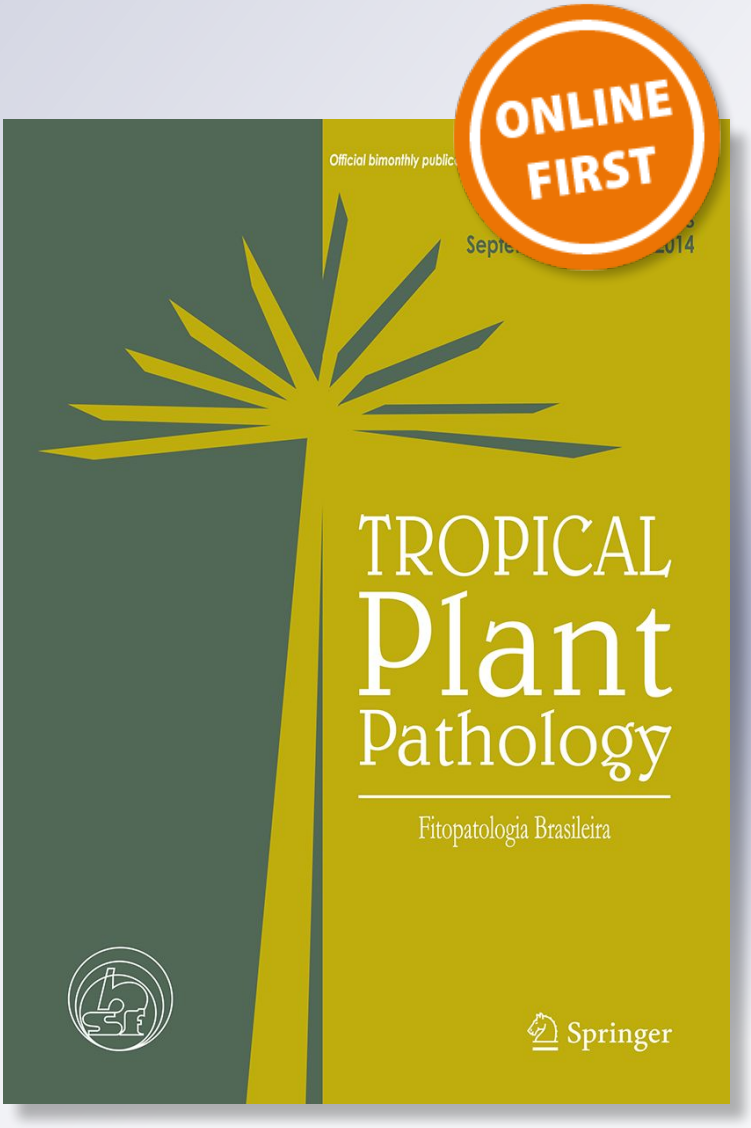

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ORIGINAL ARTICLE

Trichothecene genotypes, chemotypes and zearalenone production by *Fusarium graminearum* species complex strains causing Fusarium head blight in Argentina during an epidemic and non-epidemic season

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Abstract Members of *Fusarium graminearum* species complex are the main pathogens associated with Fusarium head blight (FHB) in wheat in Argentina, which produce trichothecene mycotoxins that are found in wheat grain and by-products. The aim of this study was to determine the chemotype and trichothecene genotypes of *Fusarium graminearum* species complex strains isolated from wheat in Argentina during an epidemic and a non-epidemic harvest season. A total of 115 and 108 strains obtained from wheat during 2012/13 and 2014/15 harvest seasons, respectively, were identified as belonging to the *Fusarium graminearum* species complex. PCR assays were used to differentiate the 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) and nivalenol (NIV) genotypes. The trichothecene type B chemotype, zearalenone, and NX-2 toxin profiles were determined based on chromatographic (LC-MS/MS) analysis. Differences in the genotype frequency were due to the target locus analyzed and year of isolation of the strain. The most common genotype and chemotype was 15-ADON in both seasons, but an increasing frequency of the 3-ADON chemotype was found in the non-epidemic season (2014/15). The strains were able to produce zearalenone and its masked derivatives, but not the type A trichothecene NX-2. This is the first report of the production of zearalenone and its modified mycotoxins by *Fusarium graminearum* species complex strains isolated from Argentina.

Keywords *Triticum aestivum* L · *Fusarium* · Chemotype · Genotype · Mycotoxins

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Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important staple food crops with an annual worldwide production of 720 million tons (FAO 2015). Wheat is the most important winter crop in Argentina, a major exporter of grain and by-products worldwide. During the 2015/16 growing season 10.9 million tons of wheat were harvested in a planted area of 3.9 million ha (BCR 2015). Fusarium head blight (FHB), one of the major diseases of wheat worldwide, affects the crop during flowering and grain filling stages under humid and warm climate conditions (McMullen et al. 2012). Members of the *Fusarium graminearum* species complex (FGSC), mainly *Fusarium graminearum* sensu stricto, are the most important FHB pathogens in South America (Magliano and Chulze 2013). The disease causes direct losses in grain yield and quality, and also threatens food safety due to contamination of grain with mycotoxins produced by the fungal pathogen. Among them, the trichothecenes such as deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) are the most important. These mycotoxins are potent inhibitors of protein synthesis and also responsible for toxic effects at neurological, gastrointestinal and immune functions (Desjardins 2006, Pestka 2010; Wu et al. 2014).

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In Argentina, FHB epidemics were recorded 17 times from 1960 to 2012 with losses in the yield estimated as high as 70% in some fields (Moschini et al. 2002; Palazzini et al. 2015). During the most recently reported epidemic season (2012/13), high levels of *F. graminearum* infection and DON contamination were observed with levels of this mycotoxin reaching up to 8.45 ppm in some samples. Weather conditions during wheat anthesis across the main regions in Argentina were highly favorable for FHB in that season, with frequent rainfall events and persistent humid conditions, which were likely responsible for reported yield losses (Moschini et al. 2013; Palazzini et al. 2015).

Given the strong dependence of FHB on seasonal weather conditions, the potential effects of global change, including climate, on plant physiology, fungal biology and the resulting disease and mycotoxin risk received increasing attention by researchers and stakeholders. For example, studies on the effect of elevated CO_2, temperatures and drought stress on growth and mycotoxin production by *Fusarium graminearum* showed the importance of the three-way interaction (Medina et al. 2015). Recent review on the effect of climate changes on wheat diseases suggests that increasing CO_2 concentration will lead to increased susceptibility of wheat to FHB (Vary et al. 2015). The continuous monitoring of genotypes and chemotypes of the FHB pathogens, especially under distinct weather conditions, is critical information for determining the risk of pathogenic and toxigenic genotypes and factors affecting their temporal and spatial distribution. Such studies have been conducted in South America during the last 15 years. In Argentina, the main phylogenetic species within FGSC associated with FHB in wheat is *F. graminearum* sensu stricto with a 15-ADON genotype (Reynoso et al. 2011; Castañares et al. 2014; Malbrán et al. 2014). However, differences in the frequency of 3-ADON producers have been reported in Argentina during 2001 (29%) and 2004 (49%) harvest seasons (Alvarez et al. 2009). The 15-ADON genotype of *F. graminearum* is also the most prevalent in Brazil, but five species are found with *F. meridionale* being an important regional contributor to FHB epidemics, such as in Paraná state (Scoz et al. 2009, Astolfi et al. 2011; Del Ponte et al. 2015). In Uruguay, regional differences in species composition have also been reported. In Eastern Uruguay, in wheat fields in a typical rice-growing region, the NIV genotype was predominant and most belonged to *F. asiaticum* (Umpiérrez-Failache et al. 2013). Other members of the FGSC were recovered from maize in Argentina including *F. meridionale* with NIV genotype and *F. boothii* with 15-ADON genotype (Sampietro et al. 2010, 2011, 2012). In Brazil, recent surveys have shown that *F. meridionale* is dominant in maize (Kuhnem et al. 2016) and *F. asiaticum* in rice (Gomes et al. 2015). In summary, *F. graminearum* 15-ADON chemotype seems to be associated with wheat, while *F. meridionale* isolates with the NIV chemotype are common on maize and *F. asiaticum* isolates with NIV chemotype on rice. These data suggest some host effect in determining the distribution of species within the FGSC. Apart from host distribution, change in climate patterns is also important driver of FGSC diversity with toxigenic potential, thus affecting the risk of mycotoxins contamination in wheat production areas worldwide (Vaughan et al. 2016).

The knowledge of the fungal population toxigenic potential is critical for predicting their impact on food safety. Therefore the aim of this study was to identify the potential and ability of FGSC strains, obtained from two growing seasons in Argentina, to produce three most common trichothecene type B, a novel trichothecene A (NX-2), and zearalenone as well as its modified toxins.

Materials and methods

Strains isolation

During the 2012/13 and 2014/15 harvest seasons, 100 wheat spikes exhibiting FHB symptoms were collected from a field at each of four different locations of the wheat growing area in Argentina (Corral de Bustos, Marcos Juárez, Carlos Pellegrini and Pergamino in 2012; and Corral de Bustos, Marcos Juárez, Carlos Pellegrini and Los Molinos in 2014) (Fig. 1). For strain isolation, one grain was taken from each symptomatic spike and plated on Nash and Snyder medium for isolation. The plates were incubated at 25 °C for 7 days under alternative

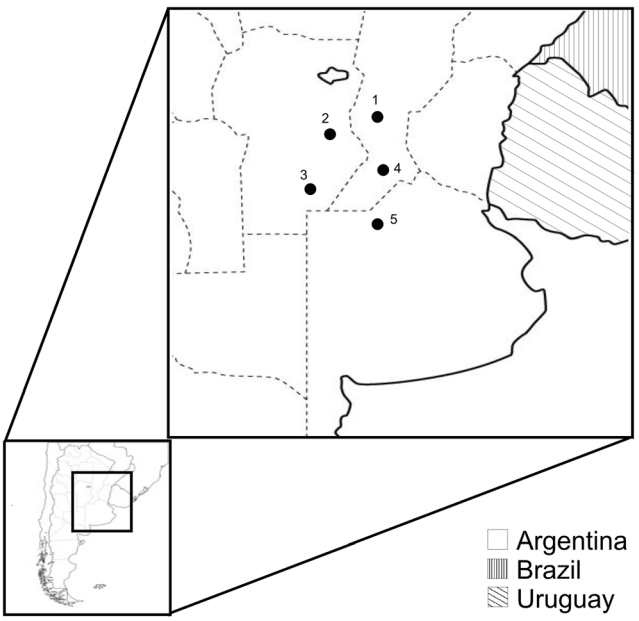

Fig. 1 Sampling area within the main wheat growing area from Argentina. Numbers 1, 2, 3 and 5 sampling fields during 2012/13 harvest season and 1, 2, 3, 4, sampling fields during 2014/15 harvest season. 1- Carlos Pellegrini (32°03′ S, 61°48′ W), 2- Marcos Juárez (32°42′ S, 62°06′ W), 3- Corral de Busto (33°17′ S, 62°12′ W), 4- Los Molinos (33°07′ S, 61°20′ W), 5- Pergamino (33°53′ S, 60°34′ W)

cycles of 12 h/12 h of white and black light, respectively. The colonies that presented characteristics of the genus *Fusarium* were transferred to Synthetic Nutritive Agar medium (SNA) and incubated under the same conditions previously described. The strains were single-spored and cultured on carnation leaf-piece agar (CLA) and potato dextrose agar (APG). Cultures were incubated for two weeks with alternate cycles of 12/12 h white/black light at 25 °C. Isolates were identified by observations on CLA medium and microscopic observation on PDA medium (Leslie and Summerell 2006).

DNA extraction

DNA was extracted using the cetyltrimethylammnonium bromide (CTAB) method from cultures grown in liquid media. In brief, isolates were grown in complete medium (CM) and incubated on an orbital shaker (150 rpm) for at least three days at 25 ± 1 °C. The resulting mycelia were harvested by filtration through non-gauze milk filters (Ken AG,). Excess water was removed by blotting mycelia between clean paper towels, and the dried mycelia were stored frozen at −20 °C until ground and extracted with CTAB (Leslie and Summerell 2006).

Trichothecene genotype determination

A multiplex PCR assay was used to determine the trichothecene genotypes 15-acetylDON (ADON), 3-ADON and nivalenol (NIV) among the FGSC isolates (Ward et al. 2008). In this assay, specific primers differentiate the genotypes based on the enzyme gene 15-O-acetyltransferase (*TRI3*) and the trichothecene efflux pump gene (*TRI12*) (Table 1). The reactions were carried out in a volume of 10 μl in 1X buffer PCR, 2 mM MgCl2, 0.2 mM of each dNTP, 0.2 μM of each primer and 0.5 U of Taq DNA Polymerase (Invitrogen,) and 100 ng of Genomic DNA. In addition, a negative control containing all reagents and primers but without the addition of DNA and as positive controls reference strains of the NIV, 3-ADON and 15-ADON genotypes were added. The amplification conditions were: 5 min at 94 °C, followed by 25 cycles of 30 s at 94 °C, 30 s at 52 °C and 1 min at 72 °C. The amplification products were resolved on 1% agarose gels.

Table 1 Primer sequences and amplified size fragments used on the PCR assays for determination of trichothecene genotypes

Locus	Primer	Sequence	bp[1]	Genotypes
TRI3	3CON	TGGCAAAGACTGGTTCAC	840 bp	NIV
	3NA	GTGCACAGAATATACGAGC	610 bp	15-ADON
	3D15A	ACTGACCCAAGCTGCCATC	243 bp	3-ADON
	3D3A	CGCATTGGCTAACACATG		
TRI12	12CON	CATGAGCATGGTGATGTC	840 bp	NIV
	12NF	TCTCCTCGTTGTATCTGG	670 bp	15-ADON
	12-15F	TACAGCGGTCGCAACTTC	410 bp	3-ADON
	12-3F	CTTTGGCAAGCCCGTGCA		

[1] Base pairs number of the amplified product

Mycotoxin analyses

Each isolate was grown in Erlenmeyer flasks (250 ml) containing 25 g of long grain rice and 10 ml of distilled water, sterilized for 15 min. at 121 °C twice during two consecutive days. Each Erlenmeyer flask containing rice was inoculated with 1 ml with a concentration of 10^5 conidia / ml, obtained from the strain growing during 10-days in SNA culture medium in Petri dishes and incubated with periods of 12/12 h of white/black light at 25 °C. The cultures were incubated for 28 days at 25 °C under dark conditions. The inoculated Erlenmeyer flasks were shacked during the first two weeks of growth to disperse the culture in the rice. After the incubation period, the content of each Erlenmeyer flask was dried in a forced air oven at 80 °C for 2 h and then stored at −20 °C until trichothecene extraction. Dried rice was ground and homogenized and 5 g were taken and mixed with 20 ml of acetonitrile/water/acetic acid (79:20:1 *v*/v/v) shaken during 1 h and then the extracts were filtered through Whatman filter paper number 1. The filtered extracts were then placed in vials and dried under heat and nitrogen gas. The extracts were analyzed to evaluate DON, 3-ADON, 15-ADON, NIV, ZEA and NX-2 toxin by tandem liquid chromatography with mass spectrometry (LC-MS / MS) according to Malachová et al. (2014).

Weather data

Weather data of 2012/13 and 2014/15 harvest seasons were obtained from Instituto de Clima y Agua – INTA Castelar, Buenos Aires, Argentina. The data were summarized as maximum and minimum temperatures (°C), relative air humidity and the accumulated rainfall (mm) during August, September, October, November and December for each season.

Results

Trichothecene genotypes and chemotypes

Out the strains isolated from different areas, 115 and 108 strains recovered during the 2011/13 and 2014/15 harvest season, respectively, were identified as belonging to the FGSC at morphological species level. The three trichothecene genotypes were detected using the primers proposed by Ward et al. (2008). During the 2012/13 and 2014/15 seasons some differences were observed between locations. At Corral de Bustos, the further west location evaluated, there was higher diversity of

genotypes than in the other locations. Also, amplifications on *TRI12* gene for the 3/15-ADON genotype was observed in a few strains belonging to the same location (Table 2).

The allocation of the toxicogenic profiles (chemotype) of each strain was done taking into account the maximum production of the acetylated derivatives by each strain (DON/15-ADON, DON/3-ADON and NIV). Most of the strains had the15-ADON chemotype but an increased number of 3-ADON chemotype (n = 39) was found among the isolates from 2014/15 harvest (Fig. 2). The production of trichothecene type A, NX-2 toxin was not observed among the strains isolated in both harvest seasons. The toxicogenic profile by LC-MS / MS of 223 isolates from both seasons showed that the mean DON level detected was 8.68 mg/kg and 10.16 mg/kg for the strains isolated during 2012/13 and 2014/15 harvest seasons, respectively. The 15-ADON production was highest among strains isolated during the 2012/13 harvest season, whereas 3-ADON production was highest among strains isolated during the 2014/15 harvest season (Table 3). The production of ZEA, ZEA-sulphate, α-ZEA and β-ZEA among the strains differed between years of isolation (Tables 3 and 4).

Seasonal weather during flowering

There was considerable variation in the amount and frequency of rainfall during flowering period for the two seasons. In the 2012/13 season, the amount of rainfall at anthesis stage (September and October) was around 392.9 mm in the Western region (Marcos Juarez), and 379.7 mm in the Eastern-South region (Pergamino). These values were higher than reports of rainfall for the same period in 2014/15 harvest season 188.9 mm in the Western region and 208 mm at the Eastern-South region at anthesis stage. The persistent humidity conditions after the flowering were more favorable for FHB development in 2012/13 harvest season compared to the 2014/15 season.

Discussion

Isolates within the *Fusarium graminearum* species complex were collected from different wheat growing areas of Argentina and varied in their trichothecene genotypes and chemotypes between the seasons. For a few strains the trichothecene genotype was incongruent with the chemotype, further suggesting the importance of determining the toxigenic ability of the strains by chemical analysis, along with genotype determination, as shown previously (Reynoso et al. 2011). The identification and trichothecene genotyping has been accelerated by the use of multilocus genotyping (MLGT) targeting the *TRI* cluster (Ward et al. 2008; Kelly et al. 2015). The difference in the proportion of 3-ADON chemotypes between the two years, although at lower frequency than 15-ADON, agrees with the results by Alvarez et al. (2009) who evaluated strains in Argentina collected during the 2002 and 2004 harvest season. Alexander et al. (2011) demonstrated that *TRI3* was not

Table 2 Trichothecene genotypes of isolates within the *Fusarium graminearum* species complex causing *Fusarium* head blight in Argentina during an epidemic and non-epidemic year

2012/13[a]								
Locus	*TRI3*				*TRI12*			
Genotype	NIV	15-ADON	3-ADON	3-/15-ADON[b]	NIV	15-ADON	3-ADON	3-/15-ADON
Pergamino (n = 32)[c]	1[d]	30	-	-	1	26	4	-
Carlos Pellegrini (n = 27)	-	26	1	-	-	25	1	1
Marcos Juarez (n = 25)	-	25	-	-	-	24	1	-
Corral de Bustos (n = 31)	2	26	2	1	2	23	2	4
Total	3	107	3	1	3	98	8	5
2014/15								
Locus	*TRI3*				*TRI12*			
Genotype	NIV	15-ADON	3-ADON	3-/15-ADON	NIV	15-ADON	3-ADON	3-/15-ADON
Los Molinos (n = 31)	-	27	-	-	-	28	-	-
Carlos Pellegrini (n = 26)	-	26	-	-	-	26	-	-
Marcos Juarez (n = 25)	1	21	-	-	1	21	-	-
Corral de Bustos(n = 26)	1	23	1	1	1	23	1	-
Total	2	97	1	1	2	98	1	-

[a] Harvest season

[b] Amplification of 3-ADON and 15-ADON genotypes

[c] Number of strain analyzed. Missing genotypes, negative for the locus

[d] Number of strains with named genotype

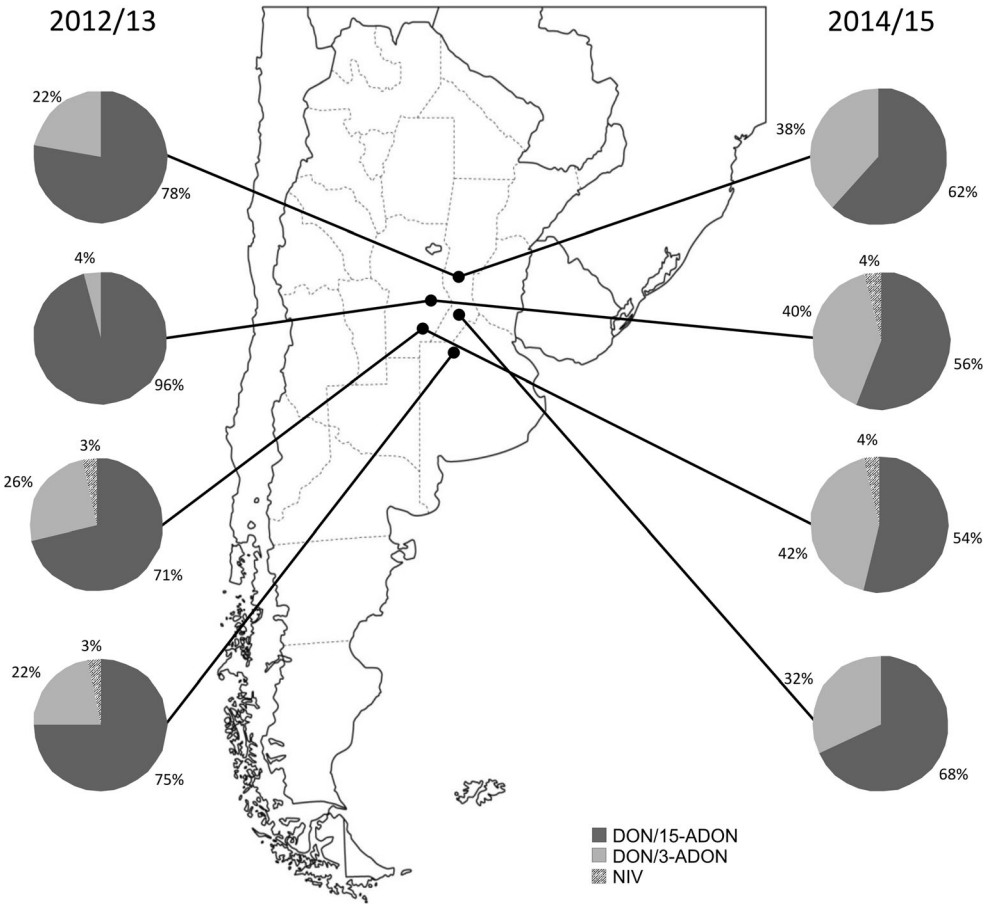

Fig. 2 Sampling areas showing the percentage of the chemotypes in the two harvest seasons evaluated

adequate to discriminate between 3-ADON and 15-ADON genotypes and showed that the *TRI8* locus is more reliable for such. It has been hypothesized that *TRI8* may have a deacetylation function in both reactions at C-3 and C-15 so it is possible that a strain DON producing can also produce 3 and 15-ADON. The differential activity of *TRI8* locus determines the differences observed between the genotypes/chemotypes

Table 3 Type B trichothecene production, analysed by LC-MS/MS, of the isolates within the *Fusarium graminearum* species complex causing *Fusarium* head blight in Argentina during an epidemic and non-epidemic year

2012/13				
Locality	DON	NIV	3-ADON	15-ADON
Pergamino (n = 32)	6.93[a]	0.08	0.47	1.68
Carlos Pellegrini (n = 27)	8.57	0.07	0.94	2.78
Marcos Juárez (n = 25)	9.54	0.07	0.62	1.61
Corral de Bustos (n = 31)	9.70	0.22	0.89	1.54
Total mean	8.68	0.11	0.73	1.90
2014/15				
Locality	DON	NIV	3-ADON	15-ADON
Los Molinos (n = 31)	10.75	0.10	0.83	0.58
Carlos Pellegrini (n = 26)	12.76	0.07	1.62	0.75
Marcos Juárez (n = 25)	11.40	0.17	2.17	1.15
Corral de Bustos (n = 26)	5.65	0.05	1.42	0.98
Total mean	10.16	0.10	1.48	0.84

[a] Mean production expressed as mg/Kg (ppm)

Table 4 Zearalenone and modified zearalenone derivatives produced by isolates within the *Fusarium graminearum* species complex causing *Fusarium* head blight in Argentina during an epidemic and non-epidemic year

2012/13	N[1]	Mean[2]	Max	Min
ZEA[3]	115/115	2.1284	6.6893	0.0224
ZEA-S	109/115	3.3313	15.5491	0.0064
αZEA	80/115	0.1919	4.5661	0.0051
βZEA	92/115	0.6415	10.4959	0.0054
2014/15	N	Mean	Max	Min
ZEA	104/108	16.2831	41.7867	0.0518
ZEA-S	103/108	14.3019	109.7067	0.0061
αZEA	99/108	0.4839	3.7493	0.0006
βZEA	103/108	5.9483	45.3867	0.0113

[1] Number of strains toxin producers / Total number of strain.

[2] Mean level of toxin production by the producer strains (mg/Kg; ppm)

[3] ZEA: Zearalenone, ZEA-S: ZEA-Sulfato. αZEA: alpha-ZEA, βZEA: beta-ZEA

(Desjardins and Proctor 2007; Alexander et al. 2011 and McCormick et al. 2013).

In this study we identified for the first time in Argentina strains from the FGSC with the ability to produce ZEA and its modified derivatives. The importance of the toxicity of ZEA modified derivatives was studied on pigs, and it was observed that these toxins were completely hydrolyzed in the gastrointestinal tract, thus contributing to the overall toxicity of ZEA (Binder et al. 2017). Liang et al. (2014) studied strains of *F. graminearum* associated with FHB and a new mycotoxin type A trichothecene, NX-2 was detected. These strains were identified in Canada and the United States of America at low frequency. The strains evaluated in our study did not produce NX 2 toxin, which agrees with the results of the genotyping assay for NX-2 for strains isolated from wheat in Argentina (Kelly et al. 2016).

The seasonal changes in chemotypes and genotypes found in our study are expected to occur due to factors related to agronomic practices or climate (van der Lee et al. 2015 and Kelly et al. 2016). This finding is relevant since changes in climate may potentially affect biodiversity and ability to cause the disease and produce secondary metabolites such as mycotoxins (Vaughan et al. 2016). For example, 3-ADON isolates of *F. graminearum* were shown to be more resilient to extreme temperature events, and in response to heat or cold become more aggressive by producing more DON and ZEA than 15-ADON isolates (Vujanovic et al. 2012). Also, the analysis of the expression of some genes of the biosynthetic pathway (*TRI*4, *TRI*5, *TRI*6, *TRI*10, *TRI*12 and *TRI*13) showed different patterns of gene expression under interacting conditions of water and temperature activity (Schmidt-Heydt et al. 2011). Continuous monitoring of changes on biodiversity, chemotypes and genotypes within FGSC populations are relevant to improve the control strategies for FHB management under a scenario of climate change.

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References

Alexander N, McCormick SP, Waalwijk C, Van der Lee T, Proctor R (2011) The genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in *Fusarium*. Fungal Genet Biol 48:485–495

Alvarez CL, Azcarate MP, Pinto VF (2009) Toxigenic potential of *Fusarium graminearum* sensu stricto isolates from wheat in Argentina. Int J Food Microbiol 135:131–135

Astolfi P, dos Santos J, Schneider L, Gomes LB, Silva CN, Tessmann DJ, Del Ponte EM (2011) Molecular survey of trichothecene genotypes of *Fusarium graminearum* species complex from barley in southern Brazil. Int J Food Microbiol 148:197–201

BCR, Bolsa de Comercio de Rosario, Informes anuales (2015/16) Available at: https://www.bcr.com.ar/Pages/gea/estAnteriores.aspx.

Binder SB, Schwartz-Zimmermann HE, Varga E, Bichl G, Michlmayr H, Adam G, Berthiller F (2017) Metabolism of Zearalenone and its major modified forms in pigs. Toxins 9:56

Castañares E, Albuquerque DR, Dinolfo MI, Pinto VF, Patriarca A, Stenglein SA (2014) Trichothecene genotypes and production profiles of *Fusarium graminearum* isolates obtained from barley cultivated in Argentina. Int J Food Microbiol 179:57–63

Del Ponte EM, Spolti P, Ward TJ, Gomes LB, Nicolli CP, Kuhnem PR, Tessmann DJ (2015) Regional and field-specific factors affect the composition of *Fusarium* head blight pathogens in subtropical no-till wheat agroecosystem of Brazil. Phytopathology 105:246–254

Desjardins AE. (2006) *Fusarium* mycotoxins: chemistry, genetics, and biology. American Phytopathological Society (APS Press)

Desjardins AE, Proctor RH (2007) Molecular biology of *Fusarium* mycotoxins. Int J Food Microbiol 119:47–50

FAO, Food and Agriculture Organization of the United Nations (2015) Crop prospects and food situation N°4. Available at: http://www.fao.org/es/Giews/english/cpfs/index.htm

Gomes LB, Ward TJ, Badiale-Furlong E, Del Ponte EM (2015) Species composition, toxigenic potential and pathogenicity of *Fusarium graminearum* species complex isolates from southern Brazilian rice. Plant Pathol 64:980–987

Kelly A, Clear RM, O'Donnell K, McCormick S, Turkington TK, Tekauz A, Ward TJ (2015) Diversity of *Fusarium* head blight populations and trichothecene toxin types reveals regional differences in pathogen composition and temporal dynamics. Fungal Genet Biol 82:22–31

Kelly A, Proctor RH, Belzile F, Chulze SN, Clear RM, Cowger C, Ward TJ (2016) The geographic distribution and complex evolutionary history of the NX-2 trichothecene chemotype from *Fusarium graminearum*. Fungal Genet Biol 95:39–48

Kuhnem PR, Ward TJ, Silva CN, Spolti P, Ciliato ML, Tessmann DJ, Del Ponte EM (2016) Composition and toxigenic potential of the *Fusarium graminearum* species complex from maize ears, stalks and stubble in Brazil. Plant Pathol 65:1185–1191

Leslie JF, Summerell BA (2006) The *Fusarium* laboratory manual. Blasckwell Professional, Ames

Liang JM, Xayamongkhon H, Broz K, Dong Y, McCormick SP, Abramova S, Kistler HC (2014) Temporal dynamics and population genetic structure of *Fusarium graminearum* in the upper Midwestern United States. Fungal Genet Biol 73:83–92

Magliano TMA, Chulze SN (2013) *Fusarium* head blight in latin America. Springer, The Netherlands

Malachová A, Sulyok M, Beltrán E, Berthiller F, Krska R (2014) Optimization and validation of a quantitative liquid chromatography–tandem mass spectrometric method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices. J Chromatogr A 1362:145–156

Malbrán I, Mourelos CA, Girotti JR, Balatti PA, Lori GA (2014) Toxigenic capacity and trichothecene production by *Fusarium graminearum* isolates from Argentina and their relationship with aggressiveness and fungal expansion in the wheat spike. Phytopathology 104:357–364

McCormick SP, Alexander NJ, Proctor RH (2013) Trichothecene triangle: toxins, genes, and plant disease. In phytochemicals, plant growth, and the environment. Springer, New York, pp 1–17

McMullen M, Bergstrom G, De Wolf E, Dill-Macky R, Hershman D, Shaner G, Van Sanford D (2012) A unified effort to fight an enemy of wheat and barley: *Fusarium* head blight. Plant Dis 96:1712–1728

Medina Á, Rodríguez A, Magan N (2015) Climate change and mycotoxigenic fungi: impacts on mycotoxin production. Curr Opin Food Sci 5:99–104

Moschini RC, Galich MTV, Annone JG, Polidoro O (2002) Fundamental-empirical approach to estimate Fusarium index in wheat. RIA J 31:39–53

Moschini RC, Martínez MI, Sepulcri MG (2013) Modeling and forecasting systems for *Fusarium* head blight and deoxynivalenol content in wheat in Argentina. In *Fusarium* Head Blight in Latin America, The Netherlands, Springer, p 205–227

Palazzini J, Fumero V, Yerkovich N, Barros G, Cuniberti M, Chulze SN (2015) Correlation between *Fusarium graminearum* and Deoxynivalenol during the 2012/13 wheat *Fusarium* head blight outbreak in Argentina. Cereal Res Comm 43:627-637

Pestka JJ (2010) Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch Toxicol 84:663–679

Reynoso MM, Ramirez ML, Torres AM, Chulze SN (2011) Trichothecene genotypes and chemotypes in *Fusarium graminearum* strains isolated from wheat in Argentina. Int J Food Microbiol 145:444–448

Sampietro DA, Marín P, Iglesias J, Presello DA, Vattuone MA, Catalan CAN, Gonzalez-Jaen MT (2010) A molecular based strategy for rapid diagnosis of toxigenic *Fusarium* species associated to cereal grains from Argentina. Fungal Biol 114:74–81

Sampietro DA, Díaz CG, Gonzalez V, Vattuone MA, Ploper LD, Catalan CA, Ward TJ (2011) Species diversity and toxigenic potential of *Fusarium graminearum* complex isolates from maize fields in northwest Argentina. Int J Food Microbiol 145:359–364

Sampietro DA, Ficoseco MEA, Jimenez CM, Vattuone MA, Catalán CA (2012) Trichothecene genotypes and chemotypes in *Fusarium graminearum* complex strains isolated from maize fields of northwest Argentina. Int J Food Microbiol 153:229–233

Schmidt-Heydt M, Parra R, Geisen R, Magan N (2011) Modelling the relationship between environmental factors, transcriptional genes and deoxynivalenol mycotoxin production by strains of two *Fusarium* species. J R Soc Interface 8:117–126

Scoz LB, Astolfi P, Reartes DS, Schmale IIIDG, Moraes MG, Del Ponte EM (2009) Trichothecene mycotoxin genotypes of *Fusarium graminearum* sensu stricto and *Fusarium meridionale* in wheat from southern Brazil. Plant Pathol 58:344–351

Umpiérrez-Failache M, Garmendia G, Pereyra S, Rodríguez-Haralambides A, Ward TJ, Vero S (2013) Regional differences in species composition and toxigenic potential among Fusarium head blight isolates from Uruguay indicate a risk of nivalenol contamination in new wheat production areas. Int J Food Microbiol 166:135–140

van der Lee T, Zhang H, van Diepeningen A, Waalwijk C (2015) Biogeography of *Fusarium graminearum* species complex and chemotypes: a review. Food Addit Contam Part A 32:453–460

Vary Z, Mullins E, Mselwain JC, Doohan F (2015) The severity of wheat disease increases whean plants and pathogens are aclimatised to elevated carbon dioxide. Glob Chang Biol 21:82–96

Vaughan M, Backhouse D, Ponte ED (2016) Climate change impacts on the ecology of *Fusarium graminearum* species complex and susceptibility of wheat to Fusarium head blight: a review. World Mycotoxin J 9(5):685–700

Vujanovic V, Goh YK, Daida P (2012) Heat-and cold-shock responses in *Fusarium graminearum* 3 acetyl-and 15 acetyl-deoxynivalenol chemotypes. J Microbiol 50:97–102

Ward TJ, Clear RM, Rooney AP, O'Donnell K, Gaba D, Patrick S (2008) An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet Biol 45:473–484

Wu F, Groopman JD, Pestka JJ (2014) Public health impacts of foodborne mycotoxins. Annu Rev Food Sci Technol 5:351–372