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SURVEY AND AFLATOXIGENIC CHARACTERIZATION FOR *ASPERGILLUS* SECTION *FLAVI* FROM THREE MAIZE PRODUCTION REGIONS OF ARGENTINA

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ABSTRACT

Argentina is one of the main exporters of maize. Soil is the main source of inoculum for the species *Aspergillus* section *Flavi* determining grain colonization and the subsequent aflatoxin production. The objective of this study was to evaluate the *Aspergillus* section *Flavi* incidence in soil and corn kernels from different regions of Argentina and evaluate the sclerotial type and the aflatoxin B_1 (AFB_1) producing capacity. Maize kernels and maize soil samples were collected at harvest from the north of Argentina and from the center and south of the province of Córdoba. Analysis was performed by comparing the distribution of culturable fungal and *Aspergillus* section *Flavi* strains. The type of sclerotia and the production of aflatoxin B_1 (AFB_1) were evaluated. The *Aspergillus* section *Flavi* counts observed in soil samples from the southern region of Córdoba were similar than those observed in samples from the central region. Severity by *Aspergillus* section *Flavi* did not exceed 9% and 2.5% in southern and northern Córdoba. 75% of the strains from the northern region of Argentina produced L sclerotia while a 25% were not sclerotia producers and showed high levels of AFB_1. The highest percentages of strains producing L sclerotia (95%) and the lowest number of S strains (5%) were isolated from the southern part of Córdoba. 61% of the strains from the central region produced L sclerotia while approximately 20% produced S sclerotia and the highest AFB_1 levels. This study contributes to the knowledge of aflatoxigenic strains from three regions of Argentina and to the development of further aflatoxin control and prevention strategies.

Key words: *Aspergillus* section *Flavi*, aflatoxin B_1, sclerotia production, Argentinean maize regions.

ANÁLISIS DE LA DISTRIBUCIÓN Y CARACTERIZACIÓN AFLATOXICOGÉNICA DE *ASPERGILLUS* SECCIÓN *FLAVI* DE TRES REGIONES MAICERAS DE ARGENTINA

RESUMEN

Argentina es uno de los principales países exportadores de maíz. El suelo constituye el principal inóculo de *Aspergillus* sección *Flavi* determinando la colonización de los granos y la posterior producción de aflatoxinas. El objetivo de este trabajo fue comparar la incidencia de hongos totales y de *Aspergillus* sección *Flavi* en suelos y granos de maíz de diferentes regiones de Argentina y evaluar el tipo de esclerocios y la capacidad de producir aflatoxina B_1 (AFB_1). Se recolectaron muestras de suelo y granos de maíz de tres regiones maiceras de Argentina, comparando la distribución fúngica y de *Aspergillus* sección *Flavi.* Se evaluó el tipo de esclerocios y la producción de AFB_1. Los recuentos de *Aspergillus* sección *Flavi* fueron similares en suelos de la región sur y centro de Córdoba. La severidad de infección en granos de maíz fue similar en el norte de Argentina y en el sur de Córdoba. La severidad por *Aspergillus* sección *Flavi* no superó el 9% y el 2,5% en el sur de Córdoba y el norte de Argentina, respectivamente. Los mayores porcentajes de cepas con esclerocios L (95%), presentaron la mayor capacidad toxicogénica, y el menor número de cepas S (5%) fueron aislados del sur de Córdoba. El 61% de las cepas de la región central de Córdoba produjeron escleróticos L, y el 20% escleróticos S con los más altos niveles de AFB_1. Este estudio contribuye al conocimiento de las cepas aflatoxigénicas presentes en Argentina, así como al desarrollo de nuevas estrategias prevención de aflatoxinas.

Palabras claves: *Aspergillus* seccion *Flavi*, Aflatoxina B_1, producción de esclerocios, regiones maiceras Argentinas.

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INTRODUCTION

Maize (*Zea mays* L.) is one of Argentina´s main crops. According to the USDA, Argentina exported nearly 17 million tons in the last harvest and ranks fourth as world exporter. During the 2015/2016 harvest, the total area sown with maize plants was estimated at nearly 4.85 million ha corresponding to different geographical areas (Bolsa de Comercio de Rosario, 2016). In Argentina, 9 regions of maize of production are recognized, which have different climatic conditions (INTA, 2016). The maize sowing period extends from September to February and the flowering usually occurs from December to March. Previous studies reported that agricultural soils, debris and insects are the main sources of *Aspergillus* section *Flavi* inocula and determine the subsequent colonization of kernels and accumulation of aflatoxins (AFs) (Nesci & Etcheverry, 2002; Nesci *et al*., 2006; Soares *et al*., 2012; Alaniz-Zanon *et al*., 2013; Perrone *et al*., 2014b). Different studies have been based on establishing the appropriate conditions for AFs production. Although several factors have been associated with increased AFs production in maize, the main determinants are drought stress, high temperatures and insects (Giorni *et al*., 2016). The great interest in knowing the development and AFs production capacities of *Aspergillus* section *Flavi* strains is based on the fact that aflatoxin B_1 (AFB_1) is the most harmful of the AFs to humans and animals (Kumar *et al.*, 2017). Based on morphological, genetic and physiological criteria, *Aspergillus flavus* can be divided into two strain types. S-type strains produce several small sclerotia and high levels of the AFs from the B series. L-type strains produce less but larger sclerotia and, in average, lower levels of AFs (Chang *et al*., 2001; Novas & Cabral, 2002). However, Razzaghi-Abyaneh *et al.* (2006) and Giorni *et al.* (2007) did not find a correlation between sclerotia size and the capacity to produce AFs. In this context, the objectives of this work was to evaluate the *Aspergillus* section *Flavi* population in maize kernels and maize soil samples collected at harvest from fields located in the north of Argentina and in the center and south of the province of Córdoba, and to evaluate the sclerotial type and the capacity to produce AFB_1 of *Aspergillus* section *Flavi* strains through the use of two culture media.

MATERIALS AND METHODS

Description of the sampling sites

The samples were collected from fields located in 4 Argentinean provinces. Three regions were sampled. Fifteen samples of maize kernels were taken during the 2014/2015 harvest from provinces of the north of Argentina (Salta, Tucumán, Santiago del Estero), and north of the province of Córdoba. In addition, fifteen soil samples were taken during the 2014/2015 harvest from the central region of Córdoba (Chucul, Carnerillo and Marcos Juarez localities). Finally, fifteen samples of maize kernels and fifteen soil samples were taken during the 2015/2016 harvest from the southern region of Córdoba (Buchardo, Serrano and San Joaquin localities). The three sampling regions were subjected to zero tillage, rotation systems for soybeans-maize and peanuts-maize crops and all the seeds used were transgenic hybrids. The north of the country is recognized as a dry and hot region, with an annual rainfall of between 500 and 750 mm and maximum temperatures ranging from 25°C to 35°C. The regions of Córdoba present annual rainfalls from 750 to 1000 mm and 1000 to 1250 mm, respectively, while the maximum temperatures range from 20°C to 25°C and from 15°C to 20°C, respectively (SMNA, 2017). The rainfall and temperatures registered in each region during the months before harvest (March to May) are presented in table 1. These months are representatives of the peak period for infection by *Aspergillus* section *Flavi*.

Maize kernels and soil sampling

From maize-growing region, 100 ears were collected at random in a diagonal direction from the lots (one lot was sampled from each locality or Province at harvest time). From each lot the ears were pooled and subsamples were taken before further analyses (Ramírez *et al.*, 1996). In the laboratory, the ears were threshed and air-dried to reduce their moisture content (to reach a 12%). Soil samples were randomly collected in a diagonal direction from all of the lots located in

the center and south of the province of Córdoba, during the same harvest periods. Each soil sample (approximately 300 g) was a pool of seven sub-samples taken from the surface layer (10 cm of depth) and at intervals of 10 m (Bududa *et al.*, 2004). In the laboratory, the samples were homogenized, air-dried at 25°C-30°C and sieved to separate soil from debris. To avoid cross-contamination with fungi and AFs between the samples, the sieve was disinfected between each sample with sodium hypochlorite (at 1%), rinsed with sterile distilled water and dried. Maize and soil samples were stored at 4°C until further analyses.

Isolation and identifcation of fungi from maize soil samples

Isolation and enumeration of culturable fungal propagules were performed using the surface spread method (Pitt & Hocking, 2009), by blending 10 g of each sample with 90 ml of 0.1% peptone water solution, then the flaks were maintained in agitation for 30 min. Serial dilutions from 10^{-1} to 10^{-4} concentrations were made. Thus, aliquots of 0.1 ml were inoculated in triplicates on Dichloran Rose Bengal Chloramphenicol agar (DRBC). The plates were incubated in darkness at 25°C for 7 days. On the last day of incubation, only plates containing 10-100 colonies were used for total and genera counting. Each colony was sub-cultured on Malt Extract Agar (MEA) for subsequent identification to the genus level (Pitt & Hocking, 2009; Samson, 2010).

Results were expressed as colony-forming units of total mycobiota and *Aspergillus* section *Flavi* per gram of soil sample (CFU g^{-1}). Yellow-green *Aspergillus* colonies, belonging to section *Flavi*, were macroscopically and microscopically characterized to differentiate them based on the morphological *A. flavus* or *A. parasiticus* groups. Strains belonging to this section were defined following the taxonomic keys described by Klich (2002), Pildain *et al*., (2005) and Samson *et al.*, (2014).

Isolation and identification of fungi from maize kernels samples

The percentages of infections were determined on Dichloran 18% Glycerol Agar (DG18) according to Perrone (2014a). Identification of the different colonies belonging to *Aspergillus* was conducted as stated above. Results were expressed as severity (percentage of infected grains) and frequency (percentage of infected samples) of fungal species and *Aspergillus* section *Flavi* strains.

Production of sclerotia

A total of 79 *Aspergillus* section *Flavi* strains were evaluated. For the sclerotia production assay, plates containing Czapek-Dox agar were inoculated with mycelia and incubated in darkness for 14 days at 30°C. Sclerotia were collected from the surfaces of plates, and the determination of sclerotia size was done according to Novas & Cabral (2002).

AFB_1 production

For screening of production capacity of aspergylic acid (Indicator of aflatoxin production potential) of members of the section *Flavi*, a qualitative assay was conducted using the medium *Aspergillus flavus-parasiticus* (AFPA) (Pitt *et al*., 1983). An orange coloration among 48-72 h on the back of the colonies indicated the possibility that the strain was able to produce AFs (Pildain *et al*., 2005).

For detection of AFB_1 production, 7-day-old cultures on MEA and Maize Meal Extract Agar (MMEA) (3% maize meal extract; 2% agar) were evaluated. The extraction of the toxin was conducted with chloroform according to Geisen (1996), with some modifications. One colony of each strain was transferred to a microtube and 500 μL of chloroform was added. The mixture was centrifuged for 20 min at 5000 rpm, the mycelium was retarded, and the chloroform extract was evaporated to dryness at room temperature. The AFB_1 in the extract was analyzed by reversed-phase HPLC/fluorescence detection system to determine the ability of these strains to produce AFB_1 (Trucksess *et al.,* 1994). The HPLC system consisted of a Hewlett-Packard 1100 pump (Palo Alto, CA, USA) connected to a Hewlett-Packard 1046 programmable fluorescence detector, interfaced to a Hewlett-Packard Chem Station. Chromatographic separations were

performed on a stainless steel Supelcosil LC-ABZ C18 reversed-phase column (150×4.6 mm i.d., 5 μL particle size; Supelco, PA, USA). Water/ methanol/ acetonitrile (4:1:1) was used as the mobile phase, at a flow rate of 1.5 ml min^{-1}. Fluorescence of aflatoxin derivatives was recorded at excitation and emission wavelengths of 360 and 440 nm, respectively. Aflatoxin B_1 standards were obtained from Sigma Chemical (St Louis, MO, USA). The detection limits for $AFB_1$was 0.0007 μg mL^{-1}.

The limits of detection (LOD), defined as the lowest concentration that the analytical process can reliably differentiate from background levels, were obtained when the signal was three times the average of background noise in the chromatogram at the lowest analyte concentration assayed. The limits of quantification (LOQ) were established as the lowest concentration assayed and validated, which gave satisfactory recovery (70-120%) and precision (<15% RSD).

Statistical analysis

Means were compared using a linear mixed model and Fisher's protected least significant difference (LSD) test to determine the significant differences between means of total fungal counts and *Aspergillus* section *Flavi* strains; and AFB_1 production. The analyses were conducted using PROC GLM InfoStat. (Universidad Nacional de Córdoba) (Di Rienzo *et. al.,* 2017)

RESULTS AND DISCUSSION

Mycobiota and *Aspergillus* section *Flavi* in maize soil and maize kernels samples

All soil and maize kernels samples showed fungal development. In maize kernels, the most prevalent genera were *Fusarium* spp*., Penicillium* spp. and *Aspergillus spp*. On the other hand, strains belonging to *Aspergillus spp. and Trichoderma spp.* were isolated more frequently from soil samples. In soil samples, total fungal and *Aspergillus* section *Flavi* strains counts ranged from 4.1 to 5.6 and from 2.0 to 4.8 $\log_{10}$ CFU g^{-1}, respectively. On the other hand, the frequency of samples contaminated with *Aspergillus* section *Flavi* was 72.7% while the severity was 4.8% in maize kernels samples (data not shown).

The average of total fungal counts in soil samples from the central region of Córdoba (5.11 $\log_{10}$ CFU g^{-1}) were higher than those observed in the sourthern region (mean 4.59 $\log_{10}$ CFU g^{-1}) ($P<0.05$). By comparison, *Aspergillus* section *Flavi* counts was similar between both regions (3.03 and 3.18 $\log_{10}$ CFU g^{-1}, respectively). Regarding the analysis in maize kernels, the severity observed in the two analyzed regions (north of Argentina and south of Córdoba) did not show significant differences between them, reaching approximately 100% of infection, and *Aspergillus* section *Flavi* severity did not exceed 2.5% and 9%, respectively ($P<0.05$) (**Figure 1**). In all of the samples, more than 96% of the strains belonging to section *Flavi* were classified within the morphological group *A. flavus* and only less than 4% corresponded to the morphological group of *A. parasiticus*.

Different research groups have reported the prevalence of *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp. in maize soils (Nesci *et al*., 2006, Carranza *et al*., 2014) and in maize and sorghum kernels (Almeida *et al*., 2006; Mahmould *et al*., 2014). In the present study in maize soils, the total fungal counts in samples from the central region of Córdoba were significantly higher than those found in the south of Córdoba. Similar results were informed by Nesci *et al.,* (2006), who analyzed maize soils from the south of Córdoba during the 2000/2001 and 2001/2002 harvests. On the other hand, our results agree with Ortega Beltran *et al.,* (2015), who suggested that the agroecological regions conditions did not influenced the structure of microbial community, from maize soil sampled during 2006 and 2008, from México. The counts of *A. flavus* detected by these authors were lower than those observed in our study and they suggest that the geographical distribution of these fungi had not influence on this capacity of produce the toxin. In the present work, the high incidence of *A. flavus* morphological group also had a homogeneous distribution in all sampled regions of maize production. In this group, there are species with the capacity to

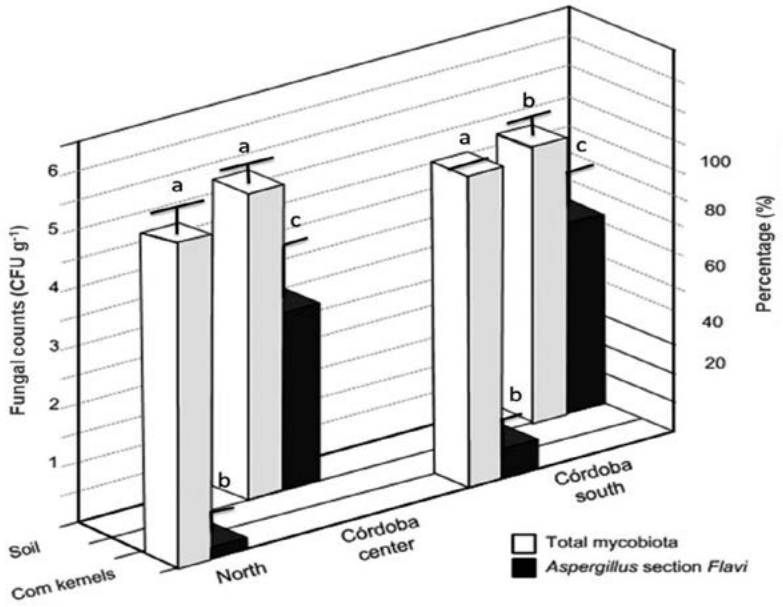

Figure 1 Total mycobiota and *Aspergillus* section *Flavi* counts (log_{10} CFU g^{-1}) in soils and severity of fungi and *Aspergillus* section *Flavi* in maize kernels from northern Argentina and from the center and south of the Córdoba Province.

Figura 1 Recuento de hongos totales y de *Aspergillus* sección *Flavi* (log_{10} CFU g^{-1}) en suelos, y severidad fúngica total de *Aspergillus* sección *Flavi* en granos de maíz del norte de Argentina y del centro y sur de la provincia de Córdoba.

produce infections in grains with the consequent deterioration of the substrate as well as the accumulation of AFs (White, 1999).

The data of frequency and severities of *Aspergillus* section *Flavi* were similar and lower respectively, than those observed by Camiletti *et al.,* (2017), who informed that all maize kernels samples collected from different geographical regions from Argentina during the 2012/2013 growing season were infected with *Aspergillus* section *Flavi*. These authors also reported a high severity (0.5% to 48.0%) in the north maize region, but similar severities by these species in maize kernels from the central region of Córdoba (0.5% to 3.5%) and from the southern maize regions of Córdoba. Also, similar frequency (42.5%) was recorded by Sebók *et al.,* (2016) on Hungarian maize from regions with comparable rainfall and temperatures regimes those observed in northern of Argentina.

In this study, no significant differences were observed between the north region of Argentina and the south of Córdoba when analyzing the infection of maize kernels by *Aspergillus* section *Flavi*. Previously, Presello & Botta (2004) assigned the variations on infection severity of *Aspergillus flavus* on maize, between growing seasons, to differences in the regimes of rainfall. The differences in climatic conditions among the sampling regions during the harvest period were not consistent with the counts of *Aspergillus* section *Flavi* in soils and maize kernels. Temperatures were similar in the three regions during the months prior to harvest, and the rainfall regimes showed more variation (**Table 1**). Under these climatic conditions, no significant differences were found in the distribution of *Aspergillus* section *Flavi* among regions. Similar results were observed in Kenya by Okoth *et al.,* (2012), who found a similar incidence of *A. flavus* in two agro-ecological zones regardless of the differences in average temperatures and rain-

Table 1. Average record of rainfall and temperature from March to May in the sampled maize producing regions.
Tabla 1. Registro promedio de lluvias y temperaturas entre marzo y mayo en las regiones productoras de maíz muestreadas.

Region	Rainfall (mm)[a]	Temperature (°C)[a]	Harvest	Samples/number of samples
North of Argentina[b]	78.3	18.42	2014/2015	Maize kernel/15
Center of Córdoba[c]	61.6	18.83	2014/2015	Soil/15
South of Córdoba[d]	53.3	16.33	2015/2016	Maize kernel/15 and soil/15

[a] Mean values.
[b] Data from automatic weather station INTA, (Cerrillos, Salta Province, http://anterior.inta.gob.ar/prorenoa/ema_cerrillos/NOAAPRYR.txt.
[c] and dData from red station weather (Bolsa de cereales de Córdoba, 2017, http://www.bccba.com.ar/dia/info/anuario-meteorologico-2015red-estaciones-meteorologicas-7501.html" y http://www.bccba.com.ar/dia/info/anuario-meteorologico-2016-7840.html

fall. Likewise, Atehnkeng *et al.,* (2008) reported similar observations when analyzing maize kernels from three agro-ecological zones in Nigeria.

Qualitative evaluation of AFB_1 production

The evaluation of the AFPA medium showed that 92% of the *Aspergillus* section *Flavi* strains produced a bright yellow-orange color in the reverse of the culture medium which is a distinctive feature of aflatoxigenic strains. Of these, 74% were shown to be AFB_1 producers on MEA, whereas 5% did not show the bright yellow-orange color feature but produced the toxin. In addition, 13% of the strains belonging to section *Flavi* did not produce the toxin not the bright yellow-orange color.

This AFPA medium was developed as a tool for a quick differentiation between potential AFs-producing *A. flavus* and *A. parasiticus* strains from several agro-ecosystems (Pitt & Hocking, 2009; Okoth *et al*., 2012). However, the occurrence of the characteristic color has also been reported by other authors in *Aspergillus* species from several substrates that do not produce AFs (Pitt & Hocking, 2009). On the other hand, Zachová *et al.,* (2003) observed a concordance between the results obtained using a molecular technique and the AFPA medium. The mentioned discrepancies allow determining that the qualitative technique is a useful tool only to carry out an initial evaluation of the toxigenic capacity of a given strain. However, results must always be complemented with more specific and sensitivity analytical techniques, such as HPLC.

Sclerotia and AFB_1 production

In this work, only the presence of AFB_1 was evaluated due to it is recognized as the main and most important, not only for its toxicity but also for its great production by *A. flavus*.

Table 2 shows the percentages of toxigenic strains and AFB_1 production for each region grouped in three ranges of production. From MEA, more than 72% of the strains were AFB_1 producers with levels from 1.38 to 208.2 $\mu g\ g^{-1}$ of mycelium. The strains with the highest and lowest producing capacity were isolated from soils of the south and center of the province of Córdoba, respectively. In MMEA, both the percentages of producing strains and the levels of AFB_1 produced were similar or lower than those observed in MEA.

In the north region of Argentina, the total strains were AFB_1 producers on MEA and more than 60% of them also produced the toxin on MMEA. In MEA, 62% of strains produced the highest levels of AFB_1, which corresponded to the maximum range of production. In MMEA, 80% of the strains corresponded to two high production ranges. The percentages of AFB_1-producing strains were lower in the center of Córdoba than in the north region of Argentina (74.5% and 61% for MEA and MMEA, respectively). Also, 97% and 85% of the strains grown on MEA and MMEA produced the lowest levels AFB_1. Finally, samples from the southern region of Córdoba showed the lowest percentages of toxin-producing strains. Indeed, 93% and 58% of the strains produced the lowest levels of AFB_1 in MEA and MMEA, respectively. The highest and lowest AFB_1 production ranges were influenced significantly by the culture medium ($P<0.05$).

During the last years, soil, maize debris and insects are considered as the main reservoirs of aflatoxigenic *Aspergillus* strains (Nesci & Etcheverry, 2002; Nesci *et al.,* 2006; Razzaghi-Abyaneh *et al.,* 2006). In these strains, MEA was the best medium for evaluating production of AFs since the levels produced by most of the strains were the highest, as compared to the MMEA medium. The levels of AFs produced by the strains isolated in the present study are similar to those reported previously from maize kernels, animal feeds based on maize and agricultural soils (Nesci & Etcheverry, 2002; Zachová *et al*., 2003; Barros *et al*., 2005; Jamali *et al*., 2012). To the best of our knowledge, only one study reported previously on the toxigenic capacity of strains isolated from maize kernels in these regions of Argentina. Camiletti *et al.,* (2017) informed on high percentages (above 90%) of *Aspergillus* section *Flavi* from the north of Argentina and the central region of Córdoba that produced AFs. These values are higher than those found in the present study. Ortega Beltran *et al.,* (2015) also reported on a 95% of

Table 2. Toxigenic capacity, based on AFB_1 production, of *Aspergillus* section *Flavi* isolated from agricultural soils and maize kernels in three maize producing regions.

Tabla 2. Capacidad toxicogénica en base a la producción de AFB_1 de *Aspergillus* sección *Flavi* aislados de suelos agrícolas y granos de maíz en tres regiones productoras.

Region	Culture medium	Positive strains/ percentage (%)[a]	AFB_1 Range ($\mu g\ g^{-1}$)	AFB_1 Mean ($\mu g\ g^{-1}$) ± SD
North of Argentina	MEA	1/12.5	0.0007 - 3.0	1.40 ± 0.00
		2/25	3.0 -10.0	5.25 ± 1.77
		5/62.5	≥ 10.0	53.01 ± 51.60
	MMEA	1/20	0.0007 - 3.0	2.7 ± 0.0
		2/40	3.0 - 10.0	4.67 ± 1.28
		2/40	≥ 10.0	15.15 ± 3.18
Center of Córdoba	MEA	28/80	0.0007 - 3.0	1.46 ± 1.30
		6/17.15	3.0 - 10.0	6.05 ± 2.17
		1/2.85	≥ 10.0	208.2 ± 0.0
	MMEA	19/67.85	0.0007 - 3.0	1.06 ± 0.78
		6/21.44	3.0 - 10.0	4.67 ± 1.53
		3/10.71	≥ 10.0	16.32 ± 4.89
South of Córdoba	MEA	13/92.85	0.0007 - 3.0	1.38 ± 0.67
		ND	3.0 - 10.0	ND
		1/7.15	≥ 10.0	61.00 ± 0.00
	MMEA	7/58.33	0.0007 - 3.0	0.91 ± 0.93
		4/33.33	3.0 - 10.0	4.47 ± 0.18
		1/8.34	≥ 10.0	105.2 ± 0.0

ND: not detected. SD: Standard deviation.
[a] Number and percentage of positive strains.
MEA: Malt Extract Agar
MMEA: Maize Meal Extract Agar
Detection limit for AFB1: 0.0007 $\mu g\ mL^{-1}$

strains from maize soils producing AFs. In contrast, Donner *et al.,* (2009) indicated that 39% of the *Aspergillus* section *Flavi* strains present in maize from Nigeria produced AFs. While Sebók *et al.,* (2016) found that nearly half of the *A. flavus* strains isolated from Hungarian maize fields were able to synthesize these toxins.

Regarding the levels of AFs and type of sclerotia produced, a great variability was observed among strains and regions. Of the total strains analyzed, 85% were able to produce sclerotia on CZ medium. Of them, 84% produced L sclerotia and only 16% produced the characteristic S sclerotia. From the north region of Argentina, 75% of the isolated strains produced large sclerotia while 25% did not produce sclerotia. Some of these strains produced high levels of AFB_1 (range 6.5 to 138.5 $\mu g\ g^{-1}$). From the Córdoba regions, the highest percentage of strains produced L sclerotia (95%). In addition, the lowest number of S strains (5%) was isolated from the south of Córdoba. The L strains showed the highest toxigenic capacity (up to 61.0 $\mu g\ g^{-1}$) in this region. Conversely, 61% of the strains isolated from the central region produced L sclerotia, while the percentages of strains producing S sclerotia or no producing sclerotia were similar (approximately 20%). In contrast to observations in the north region, the strains with the highest toxigenic capacity were those that produced S sclerotia (up to 208.7 $\mu g\ g^{-1}$). Contrarily of these results, lower percentages (63% and 57%) of L sclerotia strains were previously informed from maize and feeds based on maize (Giorni *et al*., 2007; Astoreca *et al*., 2011). Notably, L strains from several substrates worldwide

have been informed as more frequent than S strains (Nesci & Etcheverry, 2002; Pildain *et al*., 2005; Abbas *et al*., 2005; Ehrlich *et al*., 2007; Giorni *et al*.,2007; Atehnkeng *et al*., 2008; Donner *et al*., 2009; Astoreca *et al*., 2011). The occurrence of *Aspergillus* section *Flavi* in communities of maize soil in Mexico fields varied greatly during three years in all of the sampled agro-ecological regions; L type strains were dominant in 2006 and S strains increased in frequency during 2007 and 2008 (Ortega Beltran *et al*., 2015).

With respect to production of AFs and sclerotia, differences in strain ability to produce AFs have been linked to sclerotial size. A good deal of attention has been paid to the higher aflatoxigenic capacity of strains that produce small sclerotia (Chang *et al*., 2001; Novas & Cabral, 2002; Barros *et al*., 2005) whereas others reported discordant results, such as no correlation between sclerotial size and AFs production capacity, (Quinn & Keough, 2002; Giorni *et al*., 2007) or L strains and strains that do not originate sclerotia producing high levels of AFB_1 (Astoreca *et al.,* 2011). In the present study, these results allow establishing a certain relationship between the origin of the strain and its capacity to produce AFs. All of the *A. flavus* toxigenic strains from dry and high temperature zones were of the S type, while those from wet and warm zones were L type. Previous reports Jaime-Garcia *et al.,* (2006, 2010); Probst *et al.,* (2010) have informed that S strains produce, on average, more than three times the amount of AFB_1 produced by L strains. The data indicate that the relationship between the size of sclerotia and the aflatoxigenic capacity of *Aspergillus* section *Flavi* isolated from several agricultural commodities remains unclear. In this study, despite the differences in sclerotial type and AFs production between kernels samples from different regions, it is important to highlight that *Aspergillus* section *Flavi* toxigenic strains were more prevalent than non-toxigenic strains. In the same way, the high counts of these fungi in soil indicate the possibility of pre-harvest infection of kernels. Maize soils are exposed to continuous management and constitute an important reservoir for *Aspergillus* section *Flavi*. In addition, it has been suggested that the severity of contamination of a given crop in a region may be partly determined by the toxigenic potential of the strains present in such region (Horn & Dorner, 1999). Knowledge of the toxigenic strains present in each productive region can be useful for planning mycotoxins preventive practices. In Argentina, several field management strategies have been considered as promising for preventing contamination with AFs in peanuts. However, few strategies have been developed for maize (Chulze *et al*., 2015).

To conclude, this study contributes to the knowledge of aflatoxigenic strains from the north of Argentina and from the province of Córdoba and to the development of strategies for preventing contamination with AFs. Likewise, further studies to develop predictive models of AFs production by native aflatoxigenic *Aspergillus* strains under regional weather would be a valuable tool to estimate the risk of contamination after a given growing season.

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