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ORIGINAL ARTICLE

Effect of atrazine on growth and production of AFB_1 in *Aspergillus* section *Flavi* strains isolated from maize soils

Nicolás Benito [1,2] · Cecilia Soledad Carranza [1,3] · Carina Elizabeth Magnoli [1,3] · Carla Lorena Barberis [1,3]

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Abstract

Atrazine is one of the most frequently used herbicides in Argentina for controlling broadleaf weeds and annual grasses. Currently, there is limited information on the impact of triazine herbicides on mycotoxin production and growth parameters of toxigenic fungi in maize. The objective of this study was to evaluate the effect of different concentrations of atrazine on the lag phase prior to growth, the growth rate, and on production of aflatoxin B_1 (AFB_1) of *Aspergillus flavus* and *Aspergillus parasiticus* strains, on maize meal extract agar (MMEA) under different water activities (a_W) and temperatures. A commercial formulation of atrazine was added to MMEA medium at 0, 5, 10, 50, or 100 mmol/l, adjusted to 0.98, 0.95, and 0.93 a_W, and incubated at 28 °C and 37 °C for 21 days. AFB_1 was determined by HPLC after 7, 14, and 21 days of incubation. In the control treatments, a significant increase in the time prior to growth was observed and as the a_W decreased, at both temperatures, the growth rate of the strains also decreased. A significant increase in growth rate was observed as the concentration of atrazine in the medium increased, for all a_W levels tested. The optimal conditions for the accumulation of AFB_1 in the control treatments were 0.98 a_W and 28 °C, after 7 days of incubation. As the concentration of herbicide increased, AFB_1 production also increased ($P < 0.05$). These results add to the knowledge about consequences with regard to aflatoxin production of the use of excessive atrazine doses in extensive maize culture.

Keywords Atrazine · *Aspergillus flavus* · *Aspergillus parasiticus* · Water activity · Temperature · Growth parameters · Production of aflatoxin B_1

Introduction

Argentina's economy is traditionally based on agricultural production, which implies a considerable use of pesticides that have an impact on the environment (Villamil Lepori et al. 2013). Conventional tillage, reduced tillage, and no tillage are the commonly planting systems used for production of maize and soybean. Each system produces different effects on soils (Rothrock 1992; Scursoni and Satorre 2010). The use of no tillage practices, use of pesticides, and the adoption of transgenic crops designed to tolerate the pesticides have increased over the past few years (Ronco et al. 2016). The increasing productivity in agriculture has become facilitated by the use of effective chemical weed control agents, such as the s-triazine herbicides. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) was developed for the control of grassy and broadleaf weeds in maize, sugarcane, and sorghum, among other crops. During the last years, it became the most widely used herbicide worldwide due to its low cost and high effectiveness for controlling glyphosate-resistant weeds (Udiković-Kolić et al. 2012). Atrazine's moderate solubility in water, relatively low affinity for soil organic matter, and persistence in soil for months allows its transport from the soil to neighboring water bodies following rainfall events (Giddings et al. 2005). The use of atrazine has been banned in the European Union for more than 20 years now (European Commission 2004). However, atrazine has been detected in both surface and ground waters beyond authorized limits and 40% out of the total applied to crops has been detected in mature grains (De Geronimo et al. 2014; Székács et al.

✉ Carla Lorena Barberis
barberiscorreo@gmail.com

[1] Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico, Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional N° 36 Km 601, 5800 Río Cuarto, Córdoba, Argentina

[2] FONCYT (Fondo para la Investigación Científica y Tecnológica), Buenos Aires, Argentina

[3] CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Buenos Aires, Argentina

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2015). Several toxicological studies raised a great concern, since atrazine was postulated as a possible carcinogen, an endocrine disrupter, and a teratogen (Hayes et al. 2003; Lasserre et al. 2009; MacLennan et al. 2002; Wiegand et al. 2001). Sex reversal in male frogs has also been reported as an atrazine effect (Hayes et al. 2003).

Some species belonging to the *Aspergillus* genus are recognized worldwide as deterioration agents in agricultural environments (Pitt and Hocking 2009), and they can also be pathogenic for plants (Perrone et al. 2007). Their main negative trait is based on the production of metabolites (mycotoxins) that are toxic for humans and/or animals (Bennett 2010). Among these compounds, aflatoxins (AFs) are considered the most dangerous mycotoxins, which are unavoidable contaminants of food. In addition, they cannot be prevented or totally eliminated from raw materials with the current agricultural practices (Zain 2011). Aflatoxin B_1 (AFB_1) is the most toxic metabolite and is produced mainly by *A. flavus* and *A. parasiticus*. Biologically, AFB_1 behaves as immunosuppressant and inhibits phagocytosis and protein synthesis and disrupts the formation of DNA and RNA and protein synthesis in the ribosome (Carvajal 2013). For this reason, the International Agency for Research on Cancer classified it into group 1A, due to its carcinogenicity to humans. This mycotoxin is associated with liver cancer (hepatocellular carcinoma) (IARC 1993).

The growth of fungi in food products is influenced by several intrinsic, extrinsic, and processing factors. Among them, the temperature, water activity (a_W), and gas composition are considered as limiting factors for both growth and for mycotoxins production. In this context, the characterization of marginal and optimal levels of a_W and temperatures for fungal growth and mycotoxin production are essential for developing effective control strategies to reduce the incidence of mycotoxigenic fungi (Lahlali et al. 2005). The presence of xenobiotic compounds in agricultural environments also determines fungal development. Previous studies have evaluated the effects of different food grade antioxidants (Barberis et al. 2010; Passone et al. 2005) and xenobiotic agents, such as the herbicide glyphosate, on growth rate and mycotoxin production by *Aspergillus* section *Flavi* and *Nigri* (Barberis et al. 2013; Carranza et al. 2014a, b 2017; Hasan 1999a; Reddy et al. 2007). However, only limited information is available on the influence of triazine herbicides, such as atrazine, on growth and mycotoxin production of opportunistic and phytopathogenic *Aspergillus* section *Flavi* strains. The objective of this work was to evaluate the effect of different concentrations of atrazine on (i) the lag phase prior to growth, (ii) growth rates, and (iii) AFB_1 production by *Aspergillus* section *Flavi* strains under different levels of water activity and temperatures on maize-based medium.

Materials and methods

Selection of microorganisms

Three strains of *Aspergillus* section *Flavi* isolated from soil of maize producing fields, located in the south of Córdoba Province (Argentina), were selected for the ecophysiological assays. In addition, the strain *A. parasiticus* NRRL 2999 was used. The selected soil strains (*A. flavus* AFS 56, AFS 63 and *A. parasiticus* APS 55) were identified based on morphological (macroscopic and microscopic) and molecular features according to Klich (2002), Pildain et al. (2005), and Samson et al. (2010, 2014). The AFB_1 production capacity of the strains was assayed in previous works (Barberis et al. 2013). The nucleotide sequences for the ß–tubulin and calmodulin gene of *A. flavus* AFS 56 (accession numbers: MH743101–MH743108), *A. flavus* AFS 63 (accession numbers: MH743102–MH743108), and *A. parasiticus* APS 55 (accession numbers: MH743103–MH743104) strains were deposited in GenBank.

The strains were kept in glycerol (15%, Sigma-Aldrich) at − 80 °C in a culture collection at the Department of Microbiology and Immunology, National University of Río Cuarto, Córdoba, Argentina.

Herbicide

Atrazine was obtained from its commercial formulation Icona FW®, (Icona S.A., Buenos Aires, Argentina). Stock solutions (70 mg/l) were prepared by dissolving the corresponding volume of the herbicide in 100 ml of sterile distilled water (*v*/*v*). Then, the working solutions were prepared by an appropriate dilution. These solutions were sterilized with a 0.2 μm filter (Whatman, Sigma-Aldrich) and maintained at 4 °C.

Culture media

Maize meal extract agar (MMEA) (maize flour extract 3%, agar 2%) was prepared (Barberis et al. 2013). The a_W of the different culture media was adjusted to 0.98, 0.95, and 0.93 by the addition of known volumes of the non-ionic glycerol solute (Dallyn and Fox 1980). The media were sterilized in autoclave at 121 °C for 30 min. Sterilized media were temperate at 50 °C and aliquots of the atrazine solution were added to obtain the final concentrations of 0 (control), 5, 10, 50, and 100 mmol/l. Concentrations of 5 and 10 mmol/l represent atrazine levels reported in soils worldwide, while 50 and 100 mmol/l are levels reported from pesticide spill sites (Graymore et al. 2001).

The a_W of the media was confirmed at the beginning and the end of the experiment by using an Aqualab (Series 3-Decagon devices; INC., WA, USA.) in order to detect any significant deviation in this parameter.

Inoculation and incubation

Each *Aspergillus* strain was grown individually on plates containing Malt extract agar (MEA) for 7 days at 25 °C. A portion of conidia was taken and suspended on soft agar (0.1% agar). Then, this suspension was used as inoculum source (above of 10^6 spores/ml). The inoculation of the plates containing MMEA conditioned for each treatment was performed with a needle loop (2 μl) at the central point (Pitt 1975). Plates with the same a_W were placed in polyethylene bags, sealed, and incubated at 28 and 37 °C for 21 days. Each treatment was performed in triplicate and the whole assay was carried out twice.

Growth assessment

The diameter of each colony (mm) was recorded daily in two directions at an angle of 90° among them. These measures were plotted against time (days) for each strain, temperature, a_W, and atrazine concentration. The data were adjusted to a straight line corresponding to the linear growth phase, estimating from there the radial growth rate in millimeter per day. The lag phase (h) prior to growth was also determined (Barberis et al. 2010).

Total number of growth analysis was 360, resulting from three levels of a_W × two temperature levels × four *Aspergillus* strains × five treatments × three replicate experiments each.

Determination of AFB_1

After 7, 14, and 21 days of incubation, AFB_1 was determined following the methodology proposed by Geisen (1996) with some modifications. Briefly, three agar plugs were removed from different points of the center of each colony from each treatment and the toxin was extracted with 500 μl of chloroform. The mixture was centrifuged for 20 min at 5000 rpm and the chloroform extract was removed by evaporation to dryness. The extract was suspended in methanol and derivatized with trifluoroacetic acid/acetic acid/water (20:10:70). The AFB_1 in the extract was analyzed by high-performance liquid chromatography (HPLC) (Trucksess et al. 1994). Chromatographic conditions were carried out according to Barberis et al. (2013). The toxin was quantified by correlating peak areas of samples and those of standard curves. Aflatoxin B_1 standards were obtained from Sigma Chemical (St Louis, MO, USA). The detection limit of the technique for AFB_1 was 0.7 ng/g. The assay of spiking and recovery of AFB_1 from MMEA was performed according to Barberis et al. (2013).

Statistical analyses

Data of lag phases prior to growth and growth rates were transformed to log10 (x + 1) to obtain the homogeneity of variance. Means were compared by Fisher's protected LSD test to determine the influence of the factors a_W, temperature, concentration of atrazine, and strain on lag phase prior to growth, growth rate, and AFB_1 production. The analysis was conducted using PROC GLM in SAS (SAS Institute, Cary, NC) (Quinn and Keough 2002).

Results

Effect of atrazine treatments on lag phase prior to growth and on growth rate

Statistical analyses showed that strain, temperature, a_W, and atrazine concentration significantly influenced ($P < 0.05$) lag phase prior to growth and growth rate. Two, three, and four way interactions also were statistically significant for the two growth parameters evaluated (Table 1). The two strains of *A. flavus* presented a higher lag phase than the strains of *A. parasiticus* in all the tested a_W, atrazine concentrations, and temperatures. In relation to the control treatment, an increase in the lag phase with decreasing a_W (from 0.98 to 0.93) and temperature (from 37 to 28 °C) was observed for all strains. This behavior was more pronounced at 28 °C. In atrazine treatments, in general, this trend was also observed, with a slight increase in the lag phase at 0.95 and 0.93 a_W. For AFS 56 strain with 100 mol/l of atrazine at 0.98 and 0.95 a_W, the lag phase at 37 °C was lower than the lag phase observed at 28 °C.

At 28 °C, from 5 or 10 mmol/l and in all a_W, a significant decrease in lag phase was observed, compared to the control treatments, except for AF56 at 0.95 a_W, where this behavior was observed from 50 mmol/l. By comparison, at 37 °C, this decrease was observed only with 100 mmol/l, except in the case of AFS 63 at 0.98 a_W, where a significant decrease in the lag phase was observed from 50 mmol/l of atrazine ($P < 0.05$) (Table 2).

Regarding growth rate, the strains belonging to *A. flavus* showed the maximum radial growth rate in control treatments at 37 °C. On the other hand, there were no differences in *A. parasiticus* strain growth between both temperatures. Also, a parallelism between the increases in growth rate and increased a_W was observed independently of the incubation temperature. In the treatments, in general, when the amount of atrazine increased in the medium, the radial growth rate of all *Aspergillus* strains also increased significantly under all the conditions assayed ($P < 0.05$). The four strains tested had a similar behavior in their growth under all the experimental conditions. However, the two strains from the *A. parasiticus*

Table 1 Analysis of variance of the effect of water activity (a_w), temperature (T), concentration of atrazine (Conc.), and strains (I), and their interactions on lag phase prior to growth and growth rate of *A. flavus and A. parasiticus* strains

Source of variation	df[†]	Lag phase prior to growth		Growth rate	
		MS[‡]	F[§]	MS[‡]	F[§]
I	3	118,735.04	10.11*	44.22	15,713.63*
Conc.	4	17,222,239.81	1432.56*	391.43	99,999.99*
a_w	2	1,277,831.00	101.73*	42.97	13,427.22*
T	1	1,399,325.58	126.27*	1.83	637.11 *
I × Conc.	28	60,183.16	5.07*	34.71	10,561.51*
I × Conc. × a_w	78	108,771.28	9.76*	11.79	3529.32*
I × Conc. × a_w × T	119	103,112.19	8.23*	0.42	162.35*

[†] Degrees of freedom

[‡] Mean square

[§] F-Snedecor

[*] Significant $P < 0.05$

(Fig. 1c, d) showed higher growth rates than the strains of the *A. flavus* (Fig. 1a, b) at 28 °C and in all the tested a_W.

As shown in Fig. 1a, strain AFS 56 showed a significant increase ($P < 0.05$) in growth rate (92% compared to the control) with 100 mmol/l of atrazine at 0.98 a_W and 37 °C. By comparison, no significant differences were found between the treatments at 28 °C (Fig. 1b). Strain AFS 56 showed the highest growth rate at 0.95 and 0.98 a_W with 50 and 100 mmol/l of atrazine at both temperatures tested (Fig. 1c). Similarly, the strains belonging to the *A. parasiticus* strains showed the highest growth rates with 50 and 100 mmol/l of atrazine at 0.98 a_W (Fig. 1c, d).

Production of AFB_1

The statistical analyses showed that all the analyzed factors (atrazine concentrations, a_W, temperatures, and incubation time) significantly influenced ($P < 0.05$) production of AFB_1. Figures 2 and 3 show the effect of different concentrations of atrazine, a_W, temperatures, and incubation time on AFB_1 production by strains AFS 56 and APS 55, respectively. Both strains showed a similar behavior with respect to the production of AFB_1. In general, AFB_1 production did not show a similar response to that observed for growth rate. In control treatments, the highest levels of AFB_1 production varied according to the

Table 2 Effect of atrazine on lag phase prior to growth of *A. flavus* and *A. parasiticus* strains, at different a_w levels and temperatures

Strain	a_w	Lag phase prior to growth (h)									
		28 °C					37 °C				
		Atrazine (mmol/l)									
		0	5	10	50	100	0	5	10	50	100
AFS 56	0.98	31.3 ± 2.7^{no}	30.1 ± 3.3^{o}	28.2 ± 6.6^{pq}	22.3 ± 7.9^{t}	20.3 ± 5.2^{tu}	23.2 ± 3.7^{i}	23.7 ± 2.6^{i}	26.2 ± 3.1^{f}	19.5 ± 1.6^{k}	19.1 ± 2.2^{l}
	0.95	43.1 ± 1.3^{hi}	44.4 ± 5.1^{h}	47.3 ± 7.5^{fg}	32.1 ± 7.7^{h}	27.1 ± 5.9^{r}	29.2 ± 2.5^{d}	30.1 ± 3.3^{d}	27.7 ± 3.6^{f}	26.2 ± 0.9^{f}	21.5 ± 1.6^{j}
	0.93	56.2 ± 3.5^{c}	53.4 ± 2.1^{d}	50.5 ± 9.4^{e}	46.2 ± 5.1^{g}	39.2 ± 9.9^{k}	33.4 ± 4.4^{b}	32.7 ± 5.1^{b}	31.5 ± 1.7^{c}	28.6 ± 2.3^{e}	27.0 ± 4.1^{f}
AFS 63	0.98	39.2 ± 7.2^{k}	42.5 ± 8.1^{i}	37.2 ± 5.9^{m}	29.3 ± 7.7^{p}	25.2 ± 7.4^{r}	25.5 ± 3.1^{g}	23.0 ± 2.4^{i}	22.6 ± 3.3^{ij}	21.7 ± 1.2^{j}	19.3 ± 2.7^{k}
	0.95	47.3 ± 4.7^{fg}	$46.3 \pm 6,1^{g}$	38.3 ± 6.6^{l}	31.1 ± 8.4^{no}	23.1 ± 7.3^{s}	32.2 ± 3.5^{bc}	36.4 ± 5.1^{a}	33.3 ± 4.2^{b}	30.9 ± 1.3^{c}	28.9 ± 1.4^{e}
	0.93	63.2 ± 3.4^{b}	65.1 ± 6.6^{a}	57.2 ± 11^{c}	48.2 ± 10.4^{f}	41.5 ± 5.7^{j}	35.7 ± 2.6^{a}	33.2 ± 3.1^{b}	36.3 ± 0.7^{a}	29.9 ± 4.2^{d}	26.4 ± 2.1^{f}
APS 55	0.98	28.3 ± 2.1^{pq}	27.7 ± 2.1^{r}	23.7 ± 7.2^{s}	19.2 ± 5.3^{tu}	15.3 ± 5.2^{v}	19.5 ± 1.5^{k}	17.8 ± 2.1^{m}	17.3 ± 3.3^{m}	17.8 ± 0.6^{m}	16.4 ± 1.6^{n}
	0.95	32.2 ± 5.6^{n}	29.3 ± 9.5^{p}	26.1 ± 8.4^{rs}	28.8 ± 9.1^{p}	17.3 ± 3.7^{u}	23.3 ± 1.8^{i}	22.8 ± 1.7^{i}	22.5 ± 4.1^{ij}	21.9 ± 3.3^{j}	15.7 ± 0.9^{o}
	0.93	46.2 ± 9.2^{g}	48.8 ± 6.7^{f}	37.3 ± 10.4^{m}	33.2 ± 7.5^{n}	27.2 ± 9.1^{r}	24.4 ± 2.7^{h}	25.6 ± 1.9^{g}	24.9 ± 1.6^{h}	23.1 ± 4.2^{i}	19.7 ± 0.7^{k}
NRRL 2999	0.98	24.6 ± 7.1^{r}	22.7 ± 9.1^{s}	23.1 ± 5.7^{s}	19.3 ± 3.7^{tu}	11.1 ± 2.2^{z}	17.7 ± 0.6^{m}	15.6 ± 1.7^{o}	15.6 ± 2.1^{o}	14.9 ± 3.1^{p}	13.5 ± 1.6^{p}
	0.95	30.5 ± 6.9^{o}	32.4 ± 5.6^{n}	29.2 ± 5.5^{p}	23.2 ± 8.5^{s}	20.4 ± 5.6^{t}	22.7 ± 1.5^{ij}	24.6 ± 3.7^{h}	21.5 ± 2.5^{j}	21.0 ± 0.8^{j}	19.0 ± 1.3^{l}
	0.93	48.0 ± 9.8^{f}	45.2 ± 9.5^{g}	42.5 ± 8.6^{i}	39.4 ± 7.5^{k}	37.2 ± 9.3^{m}	23.8 ± 3.4^{i}	24.3 ± 2.2^{h}	25.5 ± 3.9^{g}	21.7 ± 4.1^{j}	20.6 ± 0.7^{k}

Mean values are based on quadruplicated data. Means in a row with the same letter are not significantly different according to the LSD test ($P < 0.05$). *A. flavus* strains: AFS 56 and AFS 63. *A. parasiticus* strains: APS 55 and NRRL 2999

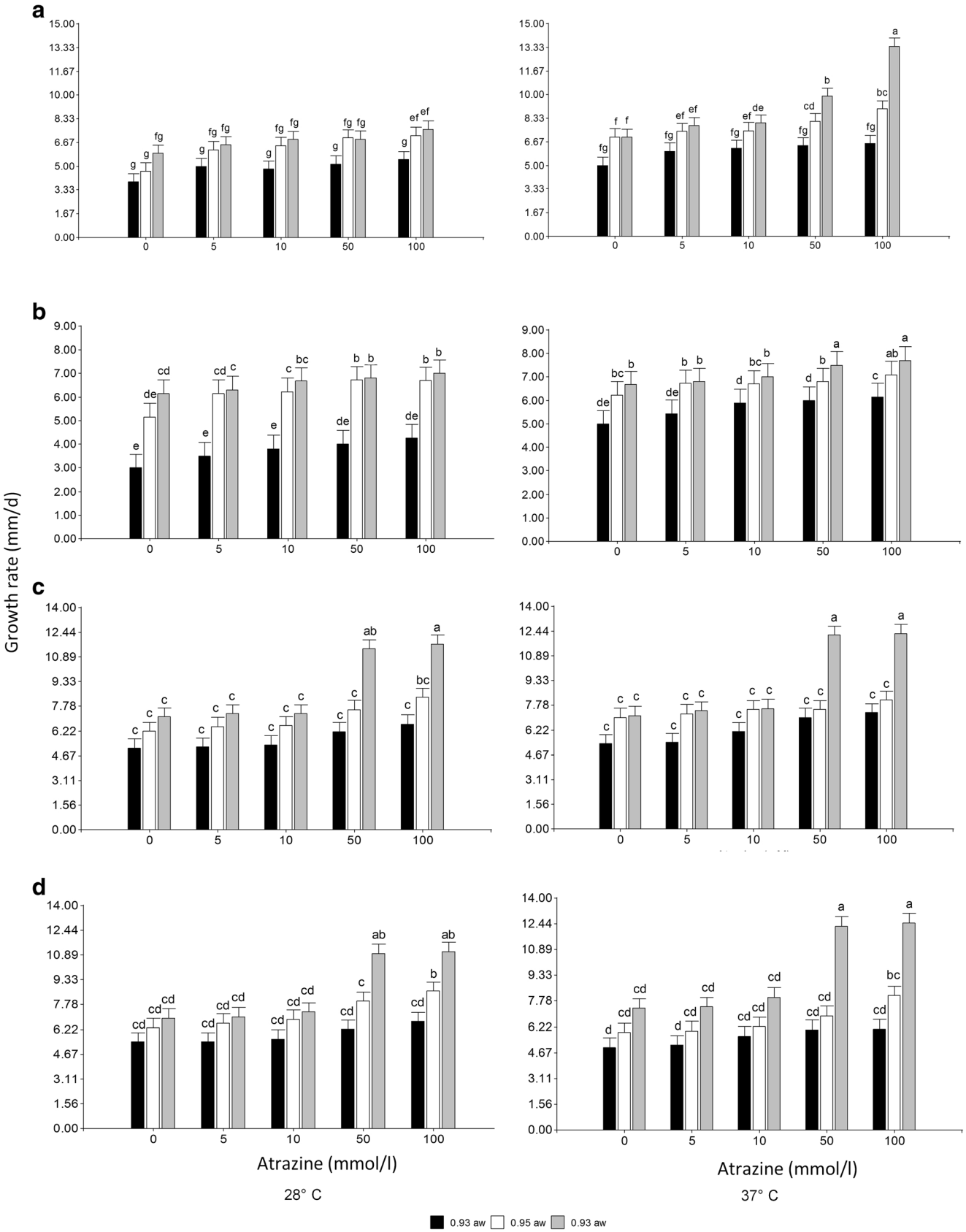

Fig. 1 Effect of atrazine on the growth rate of *A. flavus* strains (**a** and **b**), and *A. parasiticus* strains (**c** and **d**), at different a_W levels and temperatures

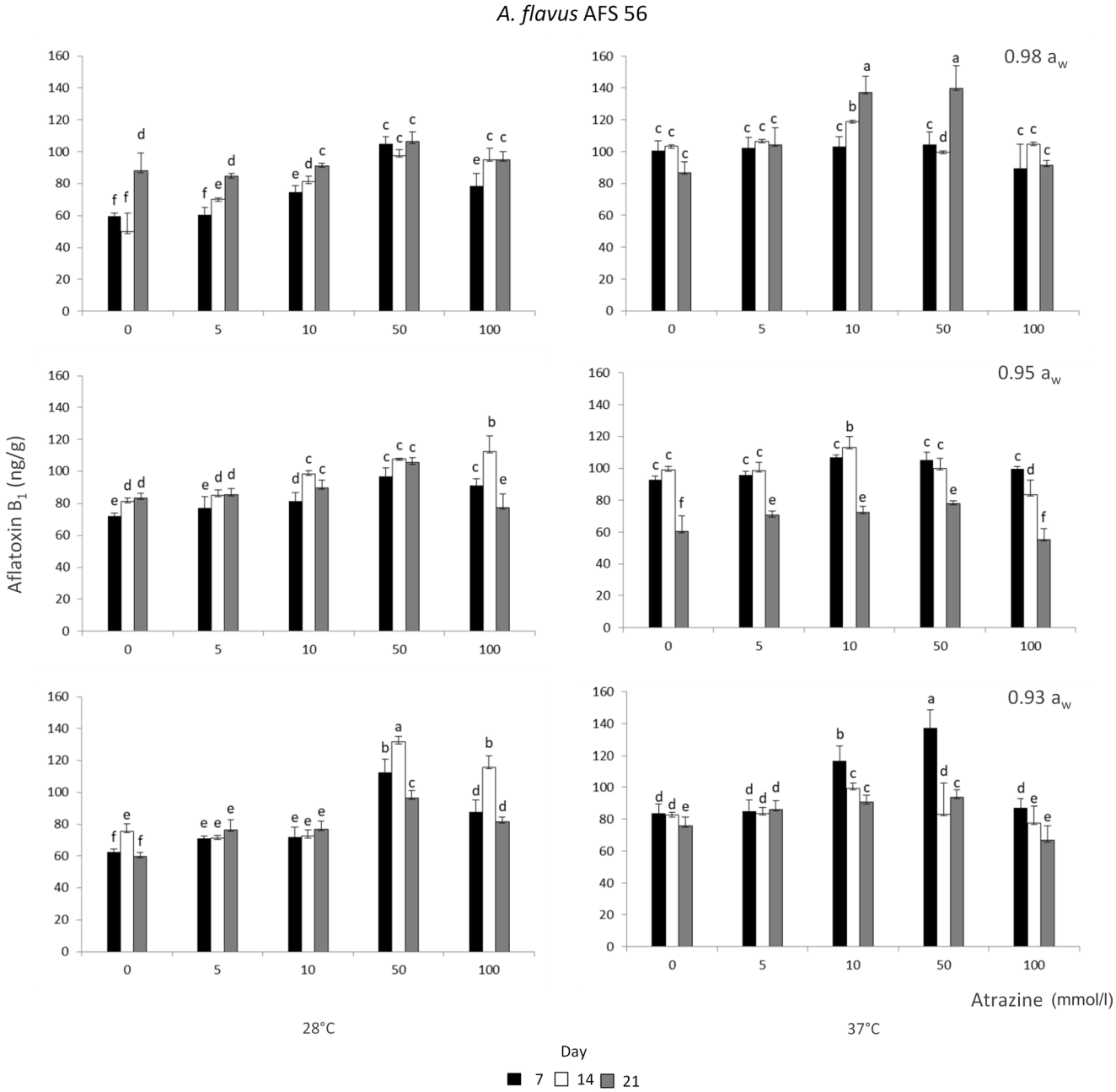

Fig. 2 Effect of atrazine on production of AFB_1 by *A. flavus* AFS 56 strain, at different a_W, temperatures, and incubation times

strain tested independently of a_W, temperature, and incubation time. In treatments with atrazine, the accumulation of AFB_1 in all strains was significantly influenced by the concentration of herbicide added to the culture medium. In general, they showed a significant increase in AFB_1 production from 10 mmol/l of atrazine, reaching the highest level with 50 mmol/l of the herbicide. In the *A. flavus* strains, this fact was observed at 37 °C (Fig. 2), while in the *A. parasiticus* strains, it was observed at 28 °C (Fig. 3). In most of the tested conditions, the strains showed a significant decrease of AFB_1 production when the concentration of atrazine increased from 50 to 100 mmol/l.

The production of AFB_1 by the strains tested with respect to the incubation time was not uniform. The highest production levels were observed in some cases at 7, 14, or 21 days independently of the herbicide treatments, a_W, and temperatures. For example, for the AFS 56 strain at 28 °C and 0.98 a_W, the maximum level of production was recorded at 21 days, while at 0.95 was recorded after 14 days of incubation, independently of the concentration of atrazine used (Fig. 2). The highest production of AFB_1 by APS 55 was observed at 28 °C, 0.98 a_W, and 7 days, while at 0.95 was observed at 21 days of incubation (Fig. 3).

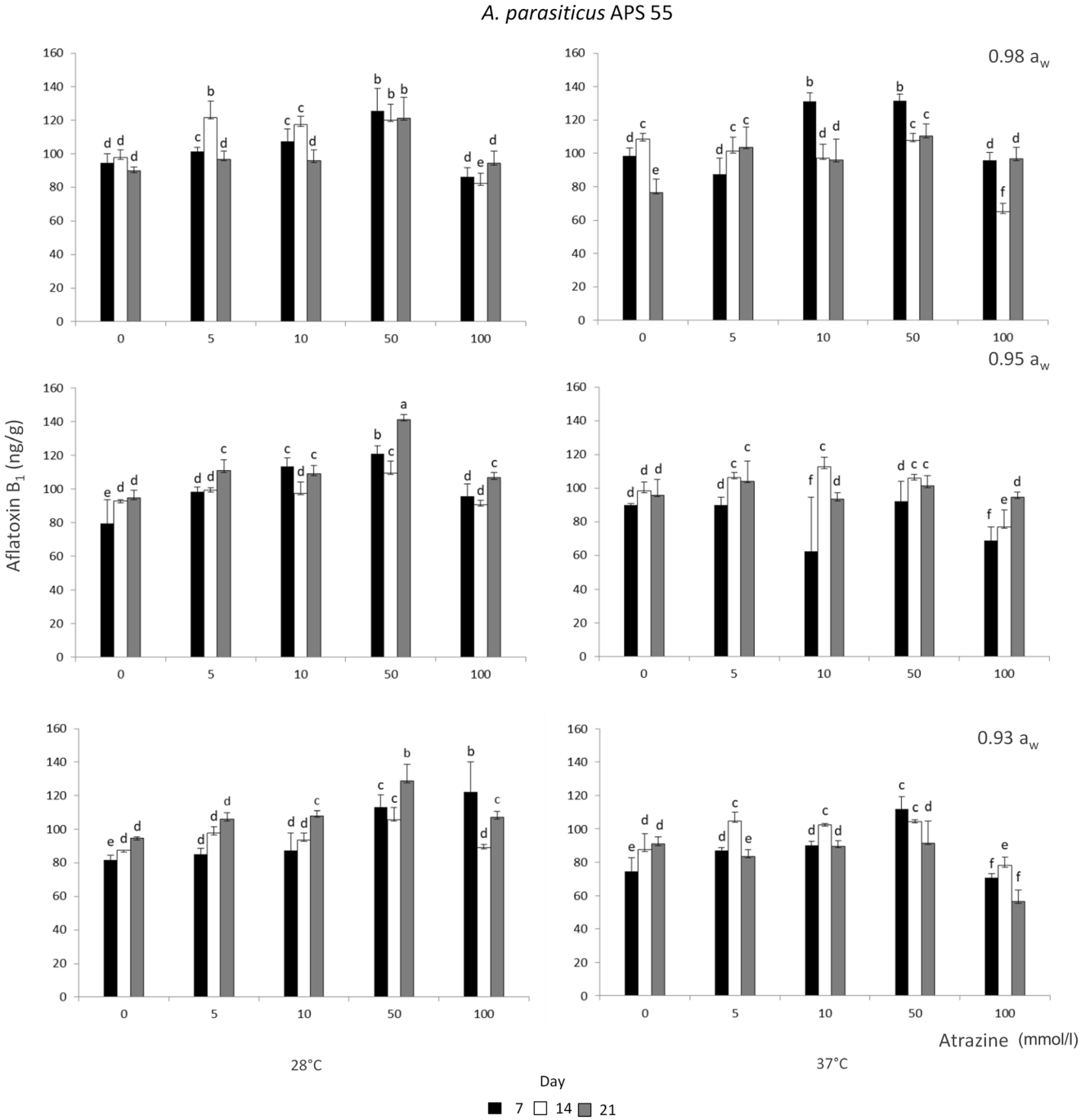

Fig. 3 Effect of atrazine on production of AFB_1 by *A. parasiticus* APS 55 strain, at different a_W, temperatures, and incubation times

Discussion

In this study, we investigated whether different concentrations of atrazine are able to decrease the time prior to growth and stimulate the growth rate and production of AFB_1 in *A. flavus and A. parasiticus* strains, isolated from maize soil, on maize meal-based medium under optimal and stressful water availability and temperature conditions. The tested temperatures were selected since both represent the mean environmental temperature detected throughout maize growth in the Córdoba region. Similarly, the water availabilities represent the optimal and stressful conditions that can be found in that environment.

Growth parameters (lag phase prior to growth and mycelial growth rate) and production of AFB_1 of strains belonging to *A. flavus* and *A. parasiticus* are significantly influenced by a_W, temperature, and atrazine concentrations and their interactions. Regarding the lag phase prior to growth, the highest

concentration of atrazine caused the most significant decrease in this parameter, at both temperatures. It is important to highlight that, at 0.98 a_W, all the tested strains except AFS 56 showed a significant decrease of this parameter from the lowest concentration of the herbicide. These results indicate that these strains were able to adapt faster to the highest herbicide concentrations compared to the controls, independently of the conditions tested. In the same way, the best conditions for the development of these aflatoxigenic fungi corresponded to those with the highest concentrations of atrazine and an optimal a_W condition (0.98). Our results showed that the presence of the herbicide in the media (5 to 100 mmol/l), under optimal incubation conditions, caused a stimulation of the growth rate.

In the literature, there are only a few studies that evaluate the effect of herbicides on growth of fungi such as *Aspergillus* section *Flavi* isolated from agricultural environments (Barberis et al. 2013; Carranza et al. 2014a; Hasan 1999a, b; Reddy et al. 2007). Most data deal with glyphosate, the most used herbicide in the last decade. To our knowledge, only one work evaluated the effects of atrazine on *Aspergillus* strains (Carranza et al. 2014b). In that study, we determined that *A. niger* aggregate-non-toxigenic strains were able to grow on soil-based medium with different concentrations of pesticides, among them atrazine (5, 10, and 20 mg/l equivalent to 23, 46, and 93 mmol/l, respectively), under several conditions of water potential (− 0.70, − 2.78, and − 7.06 MPa). In fact, we reported an increase in growth rate with 5 and 10 mg/l at − 2.78 MPa (optimal osmotic conditions). Whereas at − 7.06 MPa (osmotic stress condition), and the highest atrazine concentration, a significant reduction in this parameter was observed. Regarding time prior to growth, the strains showed a significant increase in lag phase at − 2.78 and − 7.06 MPa with all the concentrations of the herbicide. These results partially agree with our work, since the optimal water availability and the highest herbicide concentrations caused an increase in growth rate. In the same way, the reduction of the lag phase was more notable at highest atrazine concentrations in all strains.

Previous works have indicated that there is a diverse population of fungi in contaminated soils (Gopi et al. 2012; Ibiene et al. 2011). *Mucor*, *Fusarium*, *Aspergillus*, *Penicillium*, and *Trichoderma* genera have been reported in soils contaminated with heavy metals, pesticides, and hydrocarbons (Carranza et al. 2014b; Maldonado et al. 2010). Different studies reported on the use of pesticides as a nutritional source for fungal species (Carranza et al. 2017; Krzysko-Łupicka et al. 1997). Atrazine has been reported as the most difficult compound to degrade by microbial metabolism (Singh and Singh 2016). Several studies of atrazine degradation by Basidiomycete fungi were done. These studies were performed on culture solid or liquid media and on different types of soil samples (Bastos and Magan 2009; Bending et al. 2002; Entry et al. 1996; Mougin et al. 1994). Recently, Madariaga-Navarrete et al. (2017) evaluated a model of phytoremediation and bioaugmentation of atrazine with native rhizospheric microorganisms. These authors reported that, at concentrations of this herbicide (10,000 mg/l equivalent to 46,360 mmol/l) 400 times higher than those used in the present study, there was no growth inhibition of *Trichoderma* sp. in vitro. Only a few reports have demonstrated the ability of some soil fungi such as *Aspergillus* spp., *Rhizopus* spp., *Fusarium* spp., *Penicillium* spp., and *Trichoderma* spp. to degrade atrazine (Madariaga-Navarrete et al. 2017; Sene et al. 2010). Our results showed that concentrations of atrazine of 50 and 100 mmol/l significantly stimulated the growth of *A. flavus* and *A. parasiticus* strains at optimal water availabilities independently of the incubation temperature. This response would be indicating that atrazine is being used as a nutrient. These strains were isolated from soil destined to maize growth and exposed to different pesticides, which suggests that they have an intrinsic tolerance to atrazine. Nevertheless, this stimulation in growth parameters in the presence of atrazine in toxigenic strains represents a toxicological risk, since the environmental conditions assayed also promote the production of toxins. There is no information on the influence of this herbicide on production of aflatoxins. Also, there is a limited knowledge on the effects of pesticides on production of mycotoxins. In relation to production of AFB_1, the presence of atrazine in the medium (mainly 50 to 100 mmol/l) caused a significant increase in toxin production, independently of the incubation time. These results do not agree with those reported by Barberis et al. (2013), who found that AFB_1 production by *Aspergillus* section *Flavi* strains did not show detectable differences among herbicide concentrations that could occur in soils (0.5 to 10 mmol/l), at all the tested a_W. These differences could be attributed to the low concentrations of the herbicide used. In some conditions, these authors also informed a significant stimulation in the production of AFB_1 compared to controls. This fact was more significant in *A. parasiticus* strains with 1.5 to 5.0 mmol/l of glyphosate at 0.95 a_W, and in *A. flavus* strains at the same a_W condition with 1.5 mmol/l of glyphosate.

Changes in environmental conditions determine different responses in fungal growth and production of mycotoxins. The incorporation of pesticides into the environment involves a natural selection of tolerant microorganisms. These *A. flavus* and *A. parasiticus* strains that have previously been in contact with pesticides could also have been subjected to epigenetic changes in their DNA. For this reason, fungi could adapt faster to conditions influencing growth and production of AFB_1 (Ficociello et al. 2010). This suggests that the pesticide in the medium could be used by the *Aspergillus* strains as a nutritional source, stimulating their development and subsequent production of AFB_1.

The sustained use of atrazine, applied each year, together with an extended persistence, not only has caused serious pollution problems in soils and water bodies (Jablonowski et al. 2010), but has also led to a continuous exposure of the microbiota to this herbicide. Our results are important since they highlight the possible consequences of excessive doses of

atrazine in extensive maize-based cropping systems on aflatoxin production. The complete elucidation of atrazine's effects on growth and production of aflatoxins by *Aspergillus* section *Flavi* strains require further investigations under in situ conditions. The present study provides the basis for new studies on growth and production of toxins in relation to gene expression in maize exposed to herbicides and under different environmental conditions.

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Compliance with ethical standards

Conflict of interest None.

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