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Two-dimensional profiles of fumonisin B_1 production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize grain

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Abstract

This study has examined in detail the effect of temperature (7–37°C) and water availability (water activity, a_w, 0.89–0.97) on fumonisin B_1 (FB_1) production by an isolate of *Fusarium moniliforme* and *F. proliferatum* on irradiated maize grain after incubation for 28 days. The optimum conditions for *F. moniliforme* and *F. proliferatum* were 30°C at 0.97 a_w and 15°C at 0.97 a_w, respectively. The maximum concentrations were 2861 mg kg^{-1} and 17,628 mg kg^{-1} dry wt. maize grain, respectively. At marginal a_w/temperature conditions for growth (e.g. 0.89–0.91 a_w) no FB_1 was detected (< 0.1 mg kg^{-1}). A high variability was found between replicates for *F. moniliforme*, but not for *F. proliferatum*. These data were used to construct two-dimensional diagrams of all the $a_w \times$ temperature conditions favourable for FB_1 production for the first time. The data were also subjected to a polynomical regression, which demonstrated that there was a very good fit for the 15–30°C range of temperature and at 0.97 a_w. However, at marginal environmental conditions this was not possible. This suggests that it may be possible to predict within a limited environmental range the potential for significant FB_1 production.

Keywords: *Fusarium*; Fumonisins; Water activity; Temperature; Maize

1. Introduction

In recent years, *Fusarium* species belonging to Section Liseola have attracted much attention because of their ability to produce fumonisins. Among them, *Fusarium moniliforme* and *Fusarium proliferatum* are the major producers. Since the elucidation of fumonisins by Gelderblom et al. (1988) much work has been carried out on this subject. A number of surveys have shown that a high percentage of samples of corn-based feed are contaminated by fumonisins (Wilson et al., 1990; Ross et al., 1991). They have also been found in samples intended for human consumption (Sydenham et al., 1991; Pittet et

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al., 1992). From an ecological point of view, the influence of some abiotic factors on development of fumonisin producers has been established (Marín et al., 1995a, 1996). It has also been shown that water availability (water activity, a_w) and temperature are crucial in determining the extent of fumonisin production by these species (Alberts et al., 1990; LeBars et al., 1994; Marín et al., 1995b; Cahagnier et al., 1995). However, results from these previous studies are confusing, possibly due to intraspecific differences, or the different methodologies used.

Well known mycotoxins such as aflatoxins, patulin or cyclopiazonic acid have been studied in detail and the profiles for mycotoxin production by several species determined under different environmental conditions (Northolt et al., 1977, 1978; Gqaleni et al., 1996, 1997). In general, it has been shown that the range of temperatures which allows growth of toxin producers is similar to that which allows mycotoxin formation. However, the a_w range for mycotoxin production is often narrower than that for growth. Consequently, when growth is prevented or controlled, the mycotoxin contamination should be significantly reduced or inhibited.

It must also be remembered that *Fusarium* spp. do not occupy or contaminate maize and other cereals in isolation. The competing mycoflora present in maize may have a significant role in determining niche occupation and fumonisin accumulation (Marín et al., 1998a,b). Thus, a wide range of biotic and abiotic factors in the stored grain ecosystem will determine the final quality of the stored grain.

The objective of the present study was to investigate in detail the profiles of fumonisin B_1 production by one isolate each of *F. moniliforme* and *F. proliferatum* in relation to a_w (0.89–0.97) and temperature (7–37°C), and determine whether the conditions for fumonisin formation are more restricted than those for growth.

2. Materials and methods

2.1. Fungal isolates

Two isolates belonging to the *Fusarium* Liseola section, one of *Fusarium moniliforme* Sheldon (25N) and one of *F. proliferatum* (Matsushima) Nirenberg (73N), were used in these experiments. Both isolates were isolated from maize and have previously been demonstrated to be high fumonisin-producers (Sala, 1993). These isolates are held in the Food Technology Department Culture Collection of the University of Lleida, Spain.

2.2. Irradiated maize grain

Spanish dent maize grain was irradiated with 12 kGy of gamma irradiation and stored aseptically at 4°C. The grain contained no fungal infection or contamination but had retained germinative capacity. The initial water content of the grain was 13.9% ($= 0.71\ a_w$).

2.3. Inoculation and incubation of maize for fumonisin B_1 studies

Irradiated maize grains (40 g) were weighed into sterile 250-ml beakers and rehydrated to the required a_w by addition of sterile distilled water using a moisture absorption curve developed for this maize in particular. Experiments were carried out at 0.97, 0.95, 0.93, 0.91 and 0.89 a_w. Beakers of each treatment were inoculated with 0.5 ml of a microconidial spore suspension of each isolate to obtain a final concentration of 2×10^5 spores g^{-1} maize, and shaken vigorously. The inoculated irradiated maize was placed in sterile petri dishes (20 g per plate). Plates of the same a_w treatment were enclosed in closed plastic containers together with beakers of a glycerol–water solution (glycerol, Prolabo, Rectapur™, Fontenay S/bois, France) at the same a_w as the plates, to maintain constant ERH (equilibrium relative humidity) inside the chambers and incubated at 7, 10, 15, 20, 25, 30 and 37°C (±1°C). Glycerol–water solutions were prepared according to Dallyn (1978). The water activity of all media was determined with a Novasina Thermoconstanter TH200 (accuracy, $\pm 0.01\ a_w$) (Axair Ltd. Systems for Air Treatment, Pfäffikon, Switzerland). The experiment was repeated twice.

2.4. Fumonisin B_1 quantification

Samples were incubated for 28 days, and then frozen until extraction and analyses. They were extracted using a modification of the Shephard et al. (1990) method as described by Sanchis et al. (1994).

After extraction, purified sample residue was dissolved in 0.5 ml of methanol. Two hundred microlitres of *o*-phthaldialdehyde reagent (phthaldialdehyde, Merck KGaA, Darmstadt, Germany) prepared according to Shephard et al. (1990) were added to a 50-μl sample solution. Fifty μl of this solution were injected into the HPLC system (Waters; 515 HPLC pump, 717 plus Autosampler, 474 Scanning Fluorescence Detector, 746 Data Module, Waters Spherisorb® 5 μm ODS2 4.6 × 250 mm Analytical Column; Waters® Corporation, Milford, MA, USA) within 2 min of derivatization. The eluent was methanol (for liquid chromatography, Merck KGaA) + 0.1 M $NaH_2PO_4.2H_2O$ (Probus, Probus S.A., Badalona, Spain) (75 + 25) adjusted to pH 3.35 with *o*-H_3PO_4 (Prolabo, R.P. Normapur™AR). The flow rate was 1 ml min^{-1}. Reference standard of FB_1 was purchased from CSIR, Division of Food Science and Technology, Pretoria, South Africa. The limit of detection was 0.1 mg kg^{-1} and the recovery rate varied according to the following equation:

$$\text{Recovery rate (\%)} = 135.14x^{-0.1294}$$

where x = spiked FB_1 (mg kg^{-1}) with $x = \{0.1, 1, 10, 100\}$.

Simultaneously, a portion of each sample (10 g) was dried in an oven at 105°C for 17 h (International rules for seed testing, 1976), to determine the moisture content, and the fumonisin B_1 concentrations calculated on a dry matter basis.

2.5. Statistical analyses of the data

Data were first transformed by $y = \log(x + 1)$, where x is FB_1 concentration in mg kg^{-1} dry maize, in order to homogenise variance. Analyses of variance of data were carried out by using the PROC GLM procedure in the SAS System version 6.12 (SAS Institute Inc., Cary, NC, USA). Water activity and temperature were included in the program in a RANDOM instruction. As the interaction $a_w \times$ temperature was significant ($P < 0.01$), further separate analyses were done for each level of temperature or water activity. After that, non-linear regressions were carried out by using Microsoft® Excel 97, and the predictive model equation and *R*-squared obtained in each case.

3. Results

3.1. Interspecific differences

Table 1 summarises the concentrations of fumonisin B_1 produced by the two isolates under the treatment conditions used. In general, the *F. proliferatum* isolate had optimum production at 15°C, while for *F. moniliforme*, FB_1 production was maximum at higher temperatures. *F. proliferatum* produced maximum FB_1 at 0.97 a_w and 15°C (17,628 mg kg^{-1} dry wt.), while *F. moniliforme* only produced a maximum amount of 2861 mg kg^{-1} at 0.97 a_w and 30°C.

Table 1
Mean concentrations of FB_1 (mg kg^{-1}) produced in relation to temperature and a_w levels

Temp. (°C)	Species	0.89 a_w	0.91 a_w	0.93 a_w	0.95 a_w	0.97 a_w
7	*F. moniliforme*	l.d.[a]	l.d.	l.d.	l.d.	l.d.
	F. proliferatum	l.d.	l.d.	l.d.	l.d.	l.d.
10	*F. moniliforme*	l.d.	l.d.	l.d.	5.93	20.55
	F. proliferatum	l.d.	l.d.	28.44	274.21	1762.12
15	*F. moniliforme*	l.d.	l.d.	l.d.	183.99	386.91
	F. proliferatum	l.d.	l.d.	69.34	1495.82	17,627.74
20	*F. moniliforme*	l.d.	l.d.	2.73	1198.66	1795.03
	F. proliferatum	l.d.	l.d.	22.58	1334.51	14,623.87
25	*F. moniliforme*	l.d.	l.d.	0.18	966.11	1734.71
	F. proliferatum	l.d.	l.d.	32.46	686.75	8708.30
30	*F. moniliforme*	l.d.	l.d.	l.d.	319.76	2861.21
	F. proliferatum	l.d.	l.d.	32.46	125.49	73.67
37	*F. moniliforme*	l.d.	l.d.	l.d.	429.28	1.74
	F. proliferatum	l.d.	l.d.	l.d.	l.d.	l.d.

[a] l.d., below the limit of detection.

Interestingly, there was a high variability in FB_1 production by replicates of the isolate of *F. moniliforme*, while those of *F. proliferatum* were quite consistent.

Table 2 shows that statistically, both isolates had a similar response to a_w and temperature as neither $a_w \times$ isolate nor temperature $\times$ isolate interactions were significant. However, the isolates produced significantly different amounts of FB_1 ($P<0.01$).

3.2. Effect of water activity and temperature on fumonisin B_1 production

Fig. 1 details the two-dimensional effect of a_w and temperature on the production of FB_1 by both *F. moniliforme* and *F. proliferatum*. The numbers on the isopleths joining conditions at which similar levels of FB_1 are produced are shown, with dotted lines used where extrapolation has been made. Overall, FB_1 concentration increased with a_w for both species, with non-significant production at 0.89–0.91 a_w under all the temperature levels tested. Similarly, no FB_1 was produced at 7°C by both isolates, and at 37°C by the *F. proliferatum* one. However, *F. moniliforme* still yielded significant amounts of FB_1 at 37°C at the highest a_w examined (0.95–0.97 a_w). For *F. proliferatum* optimum temperature for production was 15°C, followed by 20, 25, 10 and 30°C. For *F. moniliforme* the optimum temperature varied between 20–25–30°C, then 15 and 37°C. Both a_w and temperature were statistically significant (Table 3). However, a_w was the most important factor affecting FB_1 production.

Table 4 shows the detailed analysis of variance for each separate temperature and a_w level. It is important to note the close relationship between a_w and temperature. For example, temperature only has a significant effect at ≥ 0.93 a_w for *F. proliferatum*, and none for *F. moniliforme* except for 0.97 a_w. On the other hand, a_w always has a statistically significant effect at 15–30°C for both isolates, and at 10°C for the isolate of *F. proliferatum*.

Table 2
Analysis of variance of FB_1 production on irradiated maize grain inoculated with *Fusarium* species after a 28-day incubation period. Significance of a_w, temperature (T), isolates (I), and their interactions

Factor	DF	MS	F
I	1	4.02	25.61**
a_w	4	29.46	13.81**
$a_w \times I$	4	0.68	1.47
T	6	7.07	2.60
$a_w \times T$	24	1.93	4.18**
$I \times T$	6	1.26	2.74*
$a_w \times I \times T$	24	0.46	2.94**

* Significant $P<0.05$.
** Significant $P<0.01$.

3.3. Modelling of the fumonisin B_1 production

Modelling of the fumonisin B_1 production as a function of a_w was possible over the range 10 to 30°C for *F. proliferatum* and 15 to 30°C for *F. moniliforme*, under the other marginal levels of a_w or temperature, the influence was not significant, and consequently fitting models were not used. A third-degree polynomial function was the best fitting equation in most of the cases (Table 5). The temperature factor was significant for fitting to the model under certain a_w conditions only. Figs. 2 and 3 show the fitting which was achieved using the data in relation to a_w and temperature.

4. Discussion

This study has developed detailed two-dimensional profiles of conditions which allow the production of FB_1 for the first time. These data also show clearly that environmental factors have a significant effect on the concentrations of FB_1 produced. These data can be compared with that for growth in vitro on a maize meal agar (Marín et al., 1995a) where growth minima were observed to be in the a_w and temperature of 0.90 a_w and 5–35°C. Although only one isolate of *F. moniliforme* and *F. proliferatum* were used in this study, the data do provide some useful general conclusions on the ecological parameters which influence growth and mycotoxin production.

Previous studies on the effect of a_w and temperature on mycotoxin production have been carried out predominantly on synthetic media (Northolt et al., 1977, 1978; Gqaleni et al., 1996, 1997). However, the direct assay on irradiated maize grain with retained germinative capacity may be a more reliable approach for comparison to those in bulk stored grain. Most other studies have been carried out by

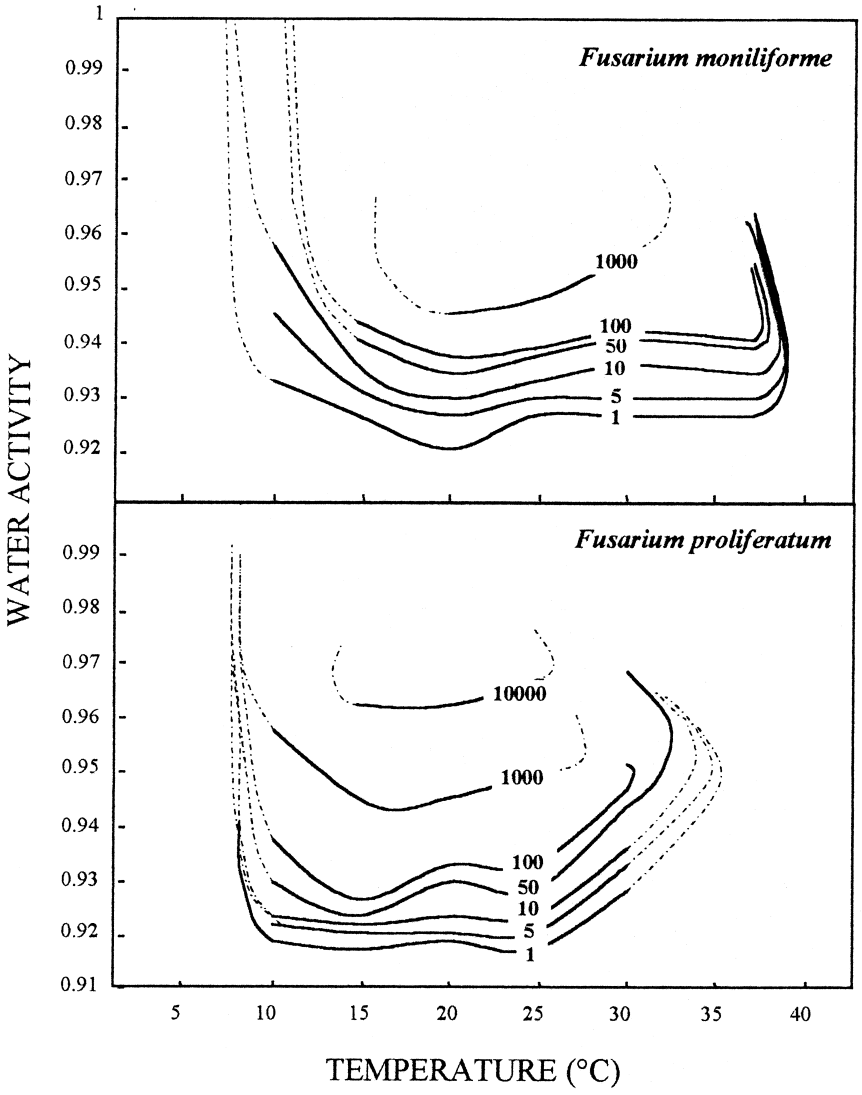

Fig. 1. Combined effect of water activity and temperature on fumonisin B_1 production (mg kg^{-1}) by *Fusarium* species after 28 days of incubation on irradiated maize.

Table 3
Analysis of variance of FB_1 production on irradiated maize grain inoculated with *Fusarium moniliforme* and *F. proliferatum* after a 28-day incubation period. Significance of a_w, temperature (T), and their interaction

Factor	DF	*F. moniliforme*		*F. proliferatum*	
		MS	*F*	MS	*F*
a_w	4	12.98	13.35**	16.97	12.17**
T	6	2.42	2.49	5.90	4.23**
$a_w \times T$	24	0.98	3.33**	1.41	71.75**

** Significant, $P<0.01$.

setting a_w and temperature to levels approximately optimum for mycotoxin production, e.g. aflatoxin (Asevedo et al., 1993; Gqaleni et al., 1997). However, in this study we have looked at limits for fumonisin production as Northolt et al. (1977) did for aflatoxins.

A 28-day incubation period was chosen in this experiment as it is a commonly used incubation time by researchers when determining fumonisin producing capacity of *Fusarium* strains: previously 15 days at 25°C plus 15 days at 15°C (Sala et al., 1994;

Table 4
Analysis of variance of FB_1 production on irradiated maize grain inoculated with *Fusarium* species after a 28-day incubation period under each separate level of a_w and temperature (T)

Level	Factor	DF	*F. moniliforme*		*F. proliferatum*	
			MS	*F*	MS	*F*
0.89	T	6	–	–	–	–
0.91	T	6	–	–	0.02	1.00
0.93	T	6	0.11	0.79	1.20	30.13*
0.95	T	6	2.53	2.30	3.72	387.1**
0.97	T	6	3.74	17.93**	4.82	164.24**
7	a_w	4	–	–	–	–
10	a_w	4	0.48	2.16	5.22	262.30**
15	a_w	4	3.43	1453.00**	6.99	1367.00**
20	a_w	4	5.16	38.79**	6.85	388.47**
25	a_w	4	5.12	54.23**	5.90	243.28**
30	a_w	4	4.01	6.38*	2.05	48.63**
37	a_w	4	0.75	0.65	0.02	0.78

* Significant, $P<0.05$.
** Significant, $P<0.01$.

Table 5
Modelling of the fumonisin B_1 production as a function of temperature and a_w

Level	*F. moniliforme*	*F. proliferatum*
0.93 a_w	–	$\log(\text{mg kg}^{-1}\ FB_1+1)=0.0004T^3-0.0422T^2+1.1207T-5.5525$; $R^2=0.94$
0.95 a_w	–	$\log(\text{mg kg}^{-1}\ FB_1+1)=0.0009T^3-0.0769T^2+1.8611T-9.0118$; $R^2=0.91$
0.97 a_w	$\log(\text{mg kg}^{-1}\ FB_1+1)=5.9104T^3-448.53T^2+9843.3T-50\,114$; $R^2=0.89$	$\log(\text{mg kg}^{-1}\ FB_1+1)=0.0006T^3-0.0468T^2+1.0109T-4.8369$; $R^2=0.83$
10°C	–	$\log(\text{mg kg}^{-1}\ FB_1+1)=-14\,602a_w^3+41\,153a_w^2-38\,585a_w+12\,037$; $R^2=0.94$
15°C	$\log(\text{mg kg}^{-1}\ FB_1+1)=-3747a_w^3+1\,048\,817a_w^2-97\,764a_w+3035$; $R^2=0.98$	$\log(\text{mg kg}^{-1}\ FB_1+1)=-30\,277a_w^3+84\,632a_w^2-78\,863a_w+24\,453$; $R^2=0.94$
20°C	$\log(\text{mg kg}^{-1}\ FB_1+1)=-5266.5a_w^3+147\,085a_w^2-1\,368\,135a_w+42\,385$; $R^2=0.97$	$\log(\text{mg kg}^{-1}\ FB_1+1)=-33\,788a_w^3+94\,622a_w^2-88\,224a_w+27\,389$; $R^2=0.96$
25°C	$\log(\text{mg kg}^{-1}\ FB_1+1)=-48\,724a_w^3+136\,186a_w^2-126\,773a_w+39\,304$; $R^2=0.98$	$\log(\text{mg kg}^{-1}\ FB_1+1)=-26\,337a_w^3+73\,796a_w^2-68\,834a_w+21\,375$; $R^2=0.96$
30°C	$\log(\text{mg kg}^{-1}\ FB_1+1)=-7708.9a_w^3+22\,366a_w^2-21\,544a_w+6892.3$; $R^2=0.83$	$\log(\text{mg kg}^{-1}\ FB_1+1)=-36\,828a_w^3+102\,865a_w^2-95\,681a_w+29\,640$; $R^2=0.77$

Sanchis et al., 1995), 21 days at 25°C (Thiel et al., 1991), and 4 weeks at 25°C have been used (LeBars et al., 1994). LeBars et al. (1994) suggested that *F. moniliforme* itself degraded the fumonisin after a period of time. This occurred ~5 weeks after incubation. Other studies suggest that this degradation begins only after 13 weeks' incubation at 20–25°C (Alberts et al., 1990).

Fumonisin B_1 concentration increased with a_w levels and was optimum at 15–30°C, with a maximum FB_1 accumulation as high as nearly 18,000 mg kg^{-1}. No FB_1 was produced at 0.89–0.91 a_w regardless of temperature level. Similarly, no FB_1 was found in samples incubated at 7°C for both isolates, and at 37°C for *F. proliferatum*. Moreover, the latter produced concentrations always <5 mg kg^{-1} at 0.93 a_w. Concentrations of >3000 mg kg^{-1}, 450 mg kg^{-1}, 10 mg kg^{-1} and 0.1 mg kg^{-1}, were obtained at 1, 0.95, 0.90 and 0.85 a_w, respectively, after a similar incubation period on autoclaved maize inocu-

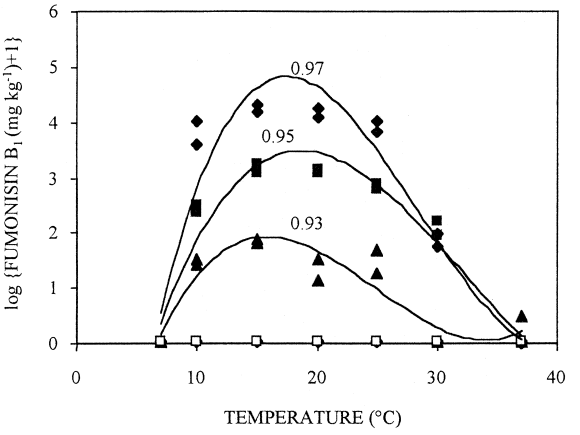

Fig. 2. Modelling of the fumonisin accumulation by *F. proliferatum* after 28 days of incubation as a function of temperature. 0.97 a_w (♦), 0.95 a_w (■), 0.93 a_w (▲), 0.91 a_w (◇), 0.89 a_w (□).

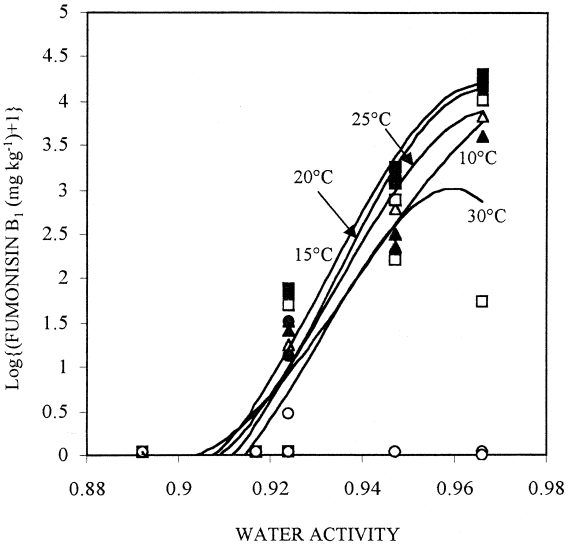

Fig. 3. Modelling of the fumonisin accumulation by *F. proliferatum* after 28 days of incubation as a function of a_w. 7°C (♦), 10°C (▲), 15°C (■), 20°C (●), 25°C (△), 30°C (□), 37°C (○).

lated with one isolate of *F. moniliforme* (Cahagnier et al., 1995). Although the incubation temperature was not reported by them, their trends parallel those obtained in the present study for *F. moniliforme*. Our *F. moniliforme* isolate had optimum production at 30–20°C, followed by 25°, 15° and 10°C. The FB_1 production rate by a *F. moniliforme* isolate on saturated autoclaved maize (1.00 a_w) (LeBars et al., 1994) was maximal at 20°C (1100 mg kg^{-1}); and decreased sharply depending on temperature in the following order: 25 (900 mg kg^{-1}), 15 (400 mg kg^{-1}), 30 (100 mg kg^{-1}), and 10°C. At 35°C, they did not detect FB_1 over the 10 weeks of the experiment. The range for growth of their strain was from 5 to >35°C. Larger amounts of about 6100 and 9300 mg kg^{-1} at 20 and 25°C, respectively were produced by a *F. moniliforme* isolate after a 4-week incubation period, but again on saturated autoclaved maize (Alberts et al., 1990). It is however important to note that FB_1 production by *F. moniliforme* isolates was subjected in our study to a low repeatability, in contrast, FB_1 production by the *F. proliferatum* isolate showed low error variability. The latter isolate produced highest concentrations of FB_1 at lower temperatures (15°C).

Grain maintained below 0.93 a_w, or at higher a_w but at low temperature (<10°C) would not allow FB_1 formation over these levels. Recent studies have demonstrated that the same isolates used in the present one were able to germinate at a minimum a_w of 0.88 and grow at 0.90 a_w in vitro. The temperature range for germination and growth was about 4 to 35°C. Optimum temperature for growth was 25–30°C and 30–37°C for germination (Marín et al., 1995a, 1996). The same isolates were used in the present study, and their ability to produce FB_1, although in small amounts, in the same temperature range for growth and germination has been demonstrated. However FB_1 production was restricted to >0.91 a_w.

Standard grain storage procedures should prevent the development of fumonisins in stored grain. Generally, fumonisin concentrations are not believed to increase during storage as long as proper conditions of grain moisture and temperature are maintained (Munkvold and Desjardins, 1997). The fact that fumonisins can be produced over 0.91 a_w shows that it is critical to avoid any delay before harvested maize is dried. Any delay would enable establishment of *Fusarium* spp. and concomitant fumonisin production. Subsequent stable storage conditions which prevent initiation and growth of spoilage species are necessary for effective long term grain quality conservation.

Interestingly, significantly higher amounts of FB_1 were obtained by using the same isolates as those used in this study on autoclaved maize (Marín et al., 1995b). However, the general trend of *F.*

moniliforme was the same, while production of FB_1 by *F. proliferatum* at 30°C was much lower than at 25°C on irradiated maize than on the autoclaved one. It must be taken into account that autoclaved maize was inoculated at a single point and consequently, after the same incubation period, the fungus had colonised a smaller amount of grain. Mycotoxin production (aflatoxin, deoxynivalenol, acetyl deoxynivalenol, zearalenone) has been demonstrated to be lower on irradiated cereals than on heat-sterilised grain. It has been suggested that this pattern of mycotoxin production is possibly caused by changes in the grain brought about by autoclaving, which favour mycotoxin production and possibly induced changes in irradiation-sterilised grain which inhibit mycotoxin production (Smith et al., 1987; O'Neill et al., 1996).

Gqaleni et al. (1997) suggested that a full factorial design experiment, as carried out in the present experiment, was useful as it allows the analysis of interactions between a range of different factors applied at different levels simultaneously and are economical and save time. In mycotoxin studies it demonstrates the complex factors controlling mycotoxin production by fungi and helps to explain their variable concentration in natural substrates. Furthermore a separate analysis of such interactions led to knowledge of the significance of a particular factor at each level, and consequently, as in the present example, enables certain data fitting to be carried out to specific models. We have previously reported on predictions of the lag phase for germination, and growth rates for *Fusarium* spp. in relation to environmental conditions (Marín et al., 1996) using Gompertz's (1825) approach. However, very little effort has been concentrated on predictive modelling of filamentous fungal growth and toxin production as has been carried out for bacteria. This may well be because of the inherent complexities associated with the quantification of fungal growth (Gibson and Hocking, 1997). Moreover, few attempts have been carried out to model mycotoxin production, except those of Pitt (1993, 1995) on aflatoxin modelling in time.

As the effect of a_w on fumonisin accumulation was much more marked, its impact was easily modelled to third-degree polynomial models under most temperature levels (10–30°C), except at 7 and 37°C where concentrations were lower regardless of a_w. In general, the differences between FB_1 production at the different temperatures tested were not high enough to provide general models, except at high a_w. Consequently, this could be a starting point to try to predict the expectable amount of FB_1 found in maize at a certain temperature and moisture content, however, the initial inoculum, time, and fluctuating environmental conditions are crucial in determining the final amount produced.

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References

Alberts, J.F., Gelderblom, W.C.A., Thiel, P.G., Marasas, W.F.O., van Schalkwyk, D.J., Behrend, Y., 1990. Effects of temperature and incubation period on production of fumonisin B_1 by *Fusarium moniliforme*. Appl. Environ. Microbiol. 56, 1729–1733.

Asevedo, I.G., Gambate, W., Correa, B., Paula, C.R., Almeida, R.M.A., Framil, V.M.S., 1993. Influence of temperature and relative humidity in the production of aflatoxins in samples of stored maize, artificially contaminated with *Aspergillus flavus* (Link). Rev. Microbiol. 24, 32–37.

Cahagnier, B., Melcion, B., Richard-Molard, D., 1995. Growth of *Fusarium moniliforme* and its biosynthesis of fumonisin B_1 on maize grain as a function of different water activities. Lett. Appl. Microbiol. 20, 247–251.

Dallyn, H., 1978. Effect of Substrate Water Activity On Growth of Certain Xerophilic Fungi. Ph.D. Thesis, South Bank University, London.

Gelderblom, W.C.A., Jaskiewicz, J., Marasas, W.F.O., Thiel, P.G., Horak, R.M., Vleggar, R., Kriek, N.P.J., 1988. Fumonisins — mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microbiol. 54, 1806–1811.

Gibson, A.M., Hocking, A.D., 1997. Advances in the predictive modelling of fungal growth in food. Trends Food Sci. Technol. 8, 353–358.

Gompertz, B., 1825. On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Phil. Trans. R. Soc. London 115, 513–585.

Gqaleni, N., Smith, J.E., Lacey, J., Gettinby, G., 1996. Production of the mycotoxin cyclopiazonic acid by *Penicillium commune*

on solid agar media: effects of water activity, temperature, and incubation time. J. Food Prot. 59, 864–868.

Gqaleni, N., Smith, J.E., Lacey, J., Gettinby, G., 1997. Effects of temperature, water activity, and incubation time on production of aflatoxins and cyclopiazonic acid by an isolate of *Aspergillus flavus* in surface agar culture. Appl. Environ. Microbiol. 63, 1048–1053.

1976. International rules for seed testing. Seed Sci. Technol. 4, 3–177.

LeBars, J., LeBars, P., Dupuy, J., Boudra, H., Cassini, R., 1994. Biotic and abiotic factors in fumonisin B_1 production and stability. J. AOAC Int. 77, 517–521.

Marín, S., Sanchis, V., Magan, N., 1995a. Water activity, temperature, and pH effects on growth of *Fusarium moniliforme* and *Fusarium proliferatum* isolates from maize. Can. J. Microbiol. 41, 1063–1070.

Marín, S., Sanchis, V., Vinas, I., Canela, R., Magan, N., 1995b. Effect of water activity and temperature on growth and fumonisin B_1 and B_2 production by *Fusarium proliferatum* and *F. moniliforme* on maize grain. Lett. Appl. Microbiol. 21, 298–301.

Marín, S., Sanchis, V., Teixido, A., Saenz, R., Ramos, A.J., Vinas, I., Magan, N., 1996. Water and temperature relations and microconidial germination of *Fusarium moniliforme* and *Fusarium proliferatum* from maize. Can. J. Microbiol. 42, 1045–1050.

Marín, S., Sanchis, V., Ramos, A.J., Viñas, I., Magan, N., 1998a. Environmental factors, in vitro interactions, and niche overlap between *Fusarium moniliforme*, *F. proliferatum*, and *F. graminearum*, *Aspergillus* and *Penicillium* species from maize grain. Mycol. Res. 102, 831–837.

Marín, S., Sanchis, V., Rull, F., Ramos, A.J., Magan, N., 1998b. Colonization of maize grain by *Fusarium moniliforme* and *Fusarium proliferatum* in the presence of competing fungi and their impact on fumonisin production. J. Food Prot. 61, 1489–1496.

Munkvold, G.P., Desjardins, A.E., 1997. Fumonisins in maize. Can we reduce their occurrence? Plant Dis. 81, 556–565.

Northolt, M.D., van Egmond, H.P., Paulsch, W.E., 1977. Differences between *Aspergillus flavus* strains in growth and aflatoxin B_1 production in relation to water activity and temperature. J. Food Prot. 40, 778–781.

Northolt, M.D., van Egmond, H.P., Paulsch, W.E., 1978. Patulin production by some fungal species in relation to water activity and temperature. J. Food Prot. 41, 885–890.

O'Neill, K., Damoglou, A.P., Patterson, M.F., 1996. The influence of gamma radiation and substrate on mycotoxin production by *Fusarium culmorum* IMI 309344. J. Appl. Bacteriol. 81, 518–524.

Pitt, R.E., 1993. A descriptive model of mold growth and aflatoxin formation as affected by environmental factors. J. Food Prot. 56, 139–146.

Pitt, R.E., 1995. A model of aflatoxin formation in stored products. Trans. ASAE 38, 1445–1453.

Pittet, A., Parisod, V., Schellenberg, M., 1992. Occurrence of fumonisins B_1 and B_2 in corn-based products from the Swiss market. J. Agric. Food Chem. 40, 1352–1354.

Ross, P.F., Rice, L.G., Plattner, R.D., Osweiler, G.D., Wilson, T.M., Owens, D.L., Nelson, H.A., Richard, J.L., 1991. Concentrations of fumonisin B_1 in feeds associated with animal health problems. Mycopathologia 114, 129–135.

Sala, N., 1993. Contaminació fúngica i de micotoxines de grans destinats a l'alimentació animal a Catalunya. Capacitat toxigènica de les soques. Ph.D. Thesis, University of Lleida, Spain.

Sala, N., Sanchis, V., Vilaro, P., Viladrich, R., Torres, M., Vinas, I., Canela, R., 1994. Fumonisin producing capacity of *Fusarium* strain isolated from cereals in Spain. J. Food Prot. 57, 915–917.

Sanchis, V., Abadias, M., Oncins, L., Sala, N., Vinas, I., Canela, R., 1994. Occurrence of fumonisins B_1 and B_2 in corn-based products from the Spanish market. Appl. Environ. Microbiol. 60, 2147–2148.

Sanchis, V., Abadias, M., Oncins, L., Sala, N., Vinas, I., Canela, R., 1995. Fumonisins B_1 and B_2 and toxigenic *Fusarium* strains in feeds from the Spanish market. Int. J. Food Microbiol. 27, 37–44.

Shephard, G.S., Sydenham, E.W., Thiel, P.G., Gelderblom, W.C.A., 1990. Quantitative determination of fumonisins B_1 and B_2 by high-performance liquid chromatography with fluorescence detection. J. Liquid Chromatogr. 13, 2077–2087.

Smith, J.E., Cuero, R.G., Lacey, J., 1987. The influence of irradiation or autoclaving of maize seeds on growth and aflatoxin production by *Aspergillus flavus*. In: BCPC MONO No. 37. Stored Product Pest Control, pp. 63–69.

Sydenham, E.W., Shephard, G.S., Thiel, P.G., Marasas, W.F.O., Stockenström, S., 1991. Fumonisin contamination of commercial corn-based human foodstuffs. J. Agric. Food Chem. 39, 2014–2018.

Thiel, P.G., Marasas, W.F.O., Sydenham, E.W., Shephard, G.S., Gelderblom, W.C.A., Nieuwenhuis, J.J., 1991. Survey of fumonisin production by *Fusarium* species. Appl. Environ. Microbiol. 57, 1089–1093.

Wilson, T.M., Ross, P.F., Rice, L.G., Osweiler, G.D., Nelson, H.A., Owens, D.L., Plattner, R.D., Reggiardo, C., Noon, T.M., Pickrell, J.W., 1990. Fumonisin B_1 levels associated with an epizootic of equine leukoencephalomalacia. J. Vet. Diagn. Invest. 2, 213–216.