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Two-dimensional environmental profiles of growth and fumonisin production by *Fusarium proliferatum* on a wheat-based substrate

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ABSTRACT

The effect of water activity (a_W; 0.995, 0.99, 0.98, 0.96, 0.94, 0.92, and 0.90), temperature (15, 25, and 30 °C), incubation time (7, 14, 21 and 28 days), and their interactions on mycelial growth and fumonisin production on wheat-based medium by three *Fusarium proliferatum* strains isolated from wheat in Argentina was evaluated. Maximum growth rates were obtained at the highest a_W (0.995) and 30 °C, with growth decreasing as the a_W of the medium was reduced. Maximum amounts of total fumonisins (FB_1, FB_2 and FB_3) were produced at 0.99 a_W and 25 °C after 21 and 28 days of incubation for 2 strains, and at 15 °C and 0.98 a_W after 28 days of incubation for the third strain. The fumonisin concentrations varied considerably depending on the a_W and temperature interactions assayed. The studied strains had different fumonisin production profiles. *F. proliferatum* ITEM 15661 and ITEM 15664 produced FB_1 and FB_2 whereas *F. proliferatum* ITEM 15654 was able to produce FB_1, FB_2 and FB_3. Interestingly, fumonisin production profiles for each particular strain were related to incubation temperatures. Fumonisins were produced from 15 to 30 °C and at a_W values of 0.92 to 0.995 after 21 to 28 days of incubation. However at 7 and 14 days of incubation small amounts of fumonisin were produced at a_W lower than 0.94. Two-dimensional profiles of a_W by temperature interactions were developed from these data to identify areas where conditions indicate a significant risk from fumonisin accumulation on wheat. Temperature and a_W conditions that resulted in fumonisin production are those found during wheat grain development (especially milk and dough stages) in the field. This study provides useful base line data on conditions representing a high and a low risk for contamination of wheat by fumonisins which is becoming of greater concern because this cereal is destined mainly for human consumption.

1. Introduction

Wheat is the most important cereal consumed by the Argentinean population. In this country human consumption of wheat manufactured products, either semolina (*Triticum turgidum* L. var. durum) or bread (*Triticum aestivum*), is much greater than for products made from other cereals (Food Balance Sheet, 2007; Pacin et al., 2012). In Argentina durum wheat is mainly used for pasta manufacture, with production reaching 604,651 tons in 2011. Pasta production reached almost 183,000 tons in 2011, and the consumption per capita was estimated at 7.9 kg/year. Common wheat production was of 15,271,000 tons in 2012 (MAGyP, 2014). It is used mostly for manufacture of bread, breakfast cereals, cookies and cupcakes. It is remarkable that wheat flour consumption in Argentina was estimated at 7.4 kg/person/month.

Fusarium species can produce a range of mycotoxins which endanger the health of both humans and animals (Sumalan et al., 2013). The main pathogen associated with *Fusarium* head blight (FHB) in common and durum wheat in Argentina is *Fusarium graminearum* sensu stricto (Lori et al., 2003; Ramirez et al., 2006b, 2007). Deoxynivalenol (DON) contamination has been reported in both wheat types (Dalcero et al., 1997; González et al., 1997; Lori et al., 2003). However Ramirez et al. (2006a) and Palacios et al. (2011) carried out two mycological surveys during non-FHB epidemic years in common wheat and durum wheat, and found that the predominant *Fusarium* species was *Fusarium proliferatum*. Natural fumonisin contamination (mainly fumonisin B_1) was reported for the first time on durum (Palacios et al., 2011) and common wheat (Cendoya et al., 2014) in Argentina during FHB non-epidemic years. The high frequency of fumonisin contamination found (more than 90%) in both kinds of wheat and also the fact that some samples evaluated in both studies exceeded the limits established for maize and sub-products for human consumption, which is 1000 ng/g in the European Union, are important to note. The occurrence of fumonisin on wheat is of concern, since the consumption of fumonisin-contaminated maize has been epidemiologically associated with esophageal cancer (Marasas, 2001) and neural tube defects in some human populations (Missmer et al., 2006). Consequently, the International

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Agency for Research on Cancer (IARC) designated FB_1 in Group 2B as "a possible carcinogenic to humans" (IARC, 2002).

Due to the importance of wheat in the Argentinean diet, and because it has been proposed, in a study in the Netherlands, that fumonisin intake occurs mainly via the intake of wheat and wheat-products (Bakker et al., 2003), it is relevant to understand the ecology of this species on wheat.

It is well known that fungal growth and mycotoxin production result from the complex interaction of several factors and, therefore, an understanding of each factor involved is essential to understand the overall process and to predict and prevent mycotoxin development (Chamley et al., 1994). Temperature and water activity (a_W) are the primary environmental factors that influence growth and mycotoxin production by fungi, including *Fusarium* species (Marín et al., 2004). While information is available on the relationship between these factors and profiles for growth and DON production by *F. graminearum* on wheat (Ramirez et al., 2006b), there is no practical information for *F. proliferatum* on this substrate.

The aim of this work was to determine the impact of a_W, temperature, and incubation time on growth and fumonisin production on wheat extract agar by three strains of *F. proliferatum* isolated from wheat in Argentina.

2. Material and methods

2.1. Fungal strains

Three *F. proliferatum* strains ITEM 15654, ITEM 15661 and ITEM 15664 (ITEM: Agri-Food Toxigenic Fungi Culture Collection of the Institute of Sciences of Food Production, CNR, Bari, Italy; http://www.ispa.cnr.it/Collection) previously isolated from wheat grains in Argentina during 2007–2008 harvest season were used. These isolates have been characterized by molecular, biological and morphological criteria (Nelson et al., 1983; Leslie and Summerell, 2006). For the molecular characterization, sequences of elongation factor (*EF-1α*), *calmodulin*, and *FUM 8* genes indicated that these isolates belong to the *Gibberella fujikuroi* species complex and were characterized as *F. proliferatum*. In order to determinate their *G. fujikuroi* mating population (MP) crossing experiments were performed (Klittich and Leslie, 1998) with standard testers as female parents and the uncharacterized field isolates as male parents. All these strains belong to *G. fujikuroi* mating population D and their mating types are MATD-1. Also, all isolates produce fumonisins (Palacios et al., 2011). Cultures were maintained in 15% glycerol at −80 °C.

2.2. Medium

Wheat, free of fumonisin contamination, was finely milled by using a Romer mill (Romer Labs Inc., Union, MO, USA). Mixtures of 2% (w/v) of milled wheat in water were prepared and 2% (w/v) agar (technical agar N° 2, Oxoid) added. The a_W of the basic medium was adjusted to 0.995, 0.99, 0.98, 0.96, 0.94, 0.92, and 0.90 by addition of different amounts of glycerol (Dallyn and Fox, 1980). The media were autoclaved at 120 °C for 20 min. Flasks of molten media were thoroughly shaken, prior to pouring into 9 cm sterile Petri dishes. The water activity of representative samples (2 of each treatment) of media was checked with an Aqualab Series 3 (Decagon Devices, Inc., WA, USA). Additional, uninoculated control plates were prepared and measured at the end of the experiment in order to detect any significant deviation of the a_W.

2.3. Inoculation, incubation, and growth assessment

Petri plates were inoculated with a 4-mm-diameter agar disk that was taken from the margin of a 7-day-old colony of each isolate grown on synthetic nutrient agar (Gerlach, and Nirenberg, 1982) at 25 °C and transferred face down to the center of each plate. Inoculated plates of the same a_W were sealed in polyethylene bags and incubated at 15, 25, and 30 °C for 28 days. A full factorial design was used where the factors were a_W, temperature and strain, and the response was growth (total number of plates: 7 a_W × 3 temperatures × 3 strains × 3 replicates).

Assessment of growth was made every day during the incubation period, and two diameters of the growing colonies were measured at right angles to each other until the colony reached the edge of the plate. Colonies radii were plotted against time, and linear regression was applied in order to obtain the growth rate (mm/day) as the slope of the line. After the incubation period, uninoculated controls and treatments were frozen for later extraction and fumonisin determination.

2.4. Determination of fumonisins

For fumonisin extraction Petri plates of each strain at different incubation periods (7, 14, 21 and 28 days) and for every a_W and temperature condition were used. Toxins were extracted with acetonitrile: water (1 : 1 v/v) by shaking the whole culture media (~20 g) and mycelia with the solvent for 30 min on an orbital shaker (150 rpm) and then filtering the extracts through filter paper (No. 4; Whatman International Ltd., Maidstone, Kent, UK). An aliquot of the extracts (1000 μL) was taken and diluted with acetonitrile: water (1: 1 v/v) as necessary for high performance liquid chromatography (HPLC) analysis. An aliquot (50 μL) of this solution was derivatized with 200 μL of an o-phthaldialdehyde (OPA) solution obtained by adding 5 mL of 0.1 M sodium tetraborate and 50 μL of 2-mercaptoethanol to 1 mL of methanol containing 40 mg of OPA (Shephard et al.,1990). The fumonisin OPA derivates (50 μL solution) were analyzed by using reversed-phase HPLC/fluorescence detection system. The HPLC system consisted of a Hewlett-Packard 1100 pump (Hewlett-Packard, Palo Alto, CA, USA) connected to a Hewlett-Packard 1046A programmable fluorescence detector and a data module Hewlett-Packard Kayak XA (HP ChemStation Rev. A.06.01). Chromatographic separations were performed on a stainless steel, C_{18} reversed-phase column (150 × 4.6 mm i.d., 5 μm particle size; Luna-Phenomenex, Torrance, CA, USA) connected to Security Guard cartridge (4 × 3 mm i.d., 5 μm particle size; Phenomenex, Torrance, CA, USA) filled with the same phase. Methanol:0.1 M sodium dihydrogen phosphate (75:25, v/v) solution adjusted to pH 3.35 with orthophosphoric acid was used as the mobile phase, at a flow rate of 1.5 mL/min. Fluorescence of the fumonisin OPA derivatives was recorded at excitation and emission wavelengths of 335 and 440 nm, respectively. Fumonisins were measured as peak heights and compared with reference standard solutions of fumonisins B_1, B_2 and B_3 (Sigma Chemical Co., St. Louis, MO, USA). A mixed acetonitrile:water (1:1, v/v) stock solution of FB_1, FB_2 and FB_3 containing 50 μg/mL of each toxin was prepared. Four mixed working calibrant solutions (0.25, 0.5, 1.0, and 2.0 μg/mL) were prepared by diluting an aliquot of the stock solution with the appropriate volume of acetonitrile:water (1:1, v/v). The retention time of FB_1, FB_3 and FB_2 was 7.5, 16.7 and 18.5 min, respectively. Appropriate dilutions of standards and/or sample extracts were made with acetonitrile/water (1:1). The detection limit of the analytical method for the three fumonisins was 1 μg/g based on the signal-to-noise ratio 3:1. Recovery experiment was performed on 2% milled maize agar spiked at levels of 1 to 10 μg/g of each fumonisin (FB_1, FB_2 and FB_3). Mean recovery ranged from 95 to 98% and 94% for FB_1, FB_2 and FB_3, respectively.

2.5. Statistical analysis

The growth rates and mycotoxin concentration were evaluated by analysis of variance (ANOVA) to determine the effect of a_W, temperature and *F. proliferatum* strains and two- and three-way interactions. When the analysis was statistically significant, the post hoc Tukey's multiple comparison procedure was used for separation of the means. Statistical significance was judged at the level $P \leq 0.01$. Statistical analysis was done using SigmaStat for Windows Version 2.03 (SPSS Inc.).

Surface response and contour map graph were produced using Sigma Plot v.10.0 (Systat Software Inc., Hounslow, London, UK),

3. Results

3.1. Effect of a_W and temperature on growth

Fig. 1 gives a diagrammatic representation of the interaction of a_W and temperature on growth rate of all *F. proliferatum* strains studied on the wheat-based media. Optimal a_W levels for growth rate ranged from 0.995 to 0.96 at 25–30 °C, with maximum growth at the highest a_W (0.995) and at 30 °C. We defined as optimal those growth rates that were above 50% of the maximum growth rates obtained. Growth of all strains decreased as water availability of the media was reduced. The three strains were able to grow at the lowest a_W tested (0.90) at 25 and 30 °C, but no growth was observed at that a_W level at 15 °C. Two of the *F. proliferatum* strains tested (ITEM 15654 and ITEM 15661) showed similar behavior at all a_W and temperature conditions evaluated. However *F. proliferatum* ITEM 15664 showed differences at 25 °C where growth rate was higher at 0.99 than at 0.995 a_W (Fig. 1). The conditions under which equivalent growth rates occurred under different environmental conditions were joined to produce contour lines to map the relative optimum and marginal conditions for growth of the *F. proliferatum* strains examined (Fig. 2).

The ANOVA of the effect of single variables (isolate, a_W, and temperature) and two- and three-way interactions revealed that all variables alone and all interactions had a significant effect on growth rates (Table 1).

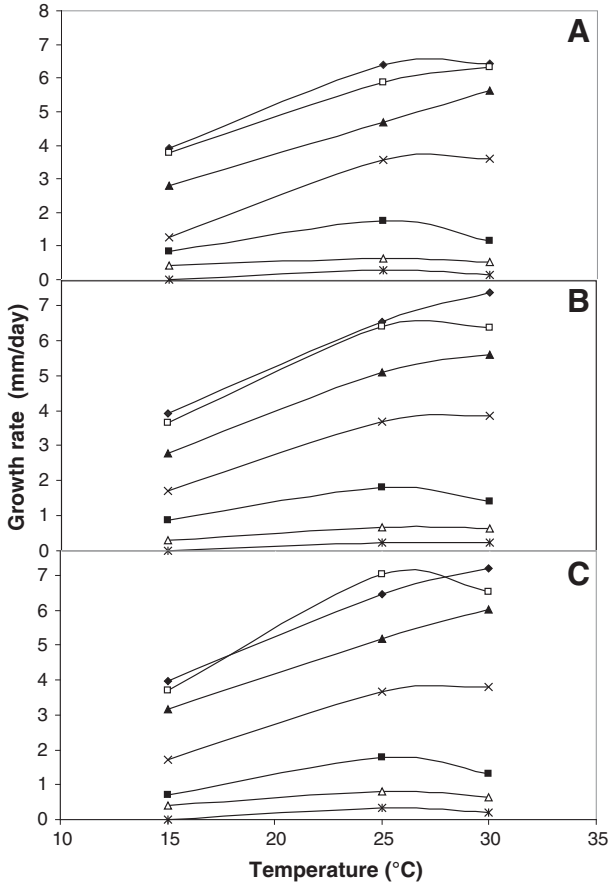

Fig. 1. Effect of water activity, 0.90 (⚹), 0.92 (Δ), 0.94 (■), 0.96 (X), 0.98 (▲), 0.99 (□), 0.995 (♦) and temperature on growth rate of three *Fusarium proliferatum* strains on wheat-based media (A: ITEM 15654; B: ITEM 15661 and C: ITEM 15664).

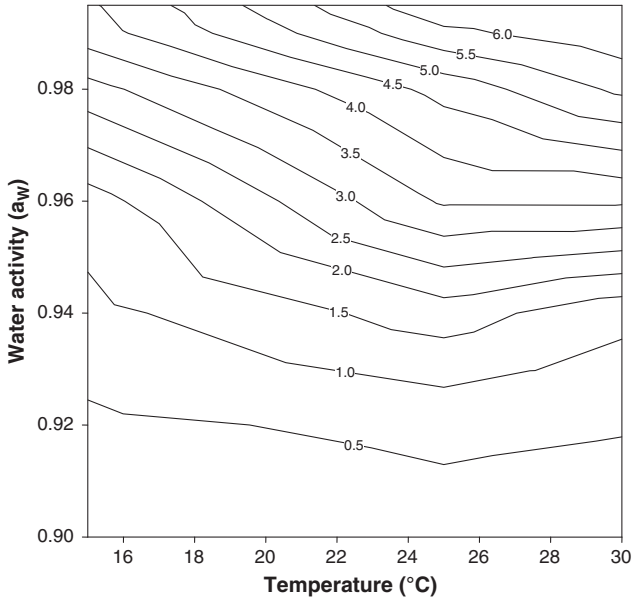

Fig. 2. Two-dimensional contour map of growth profile of *Fusarium proliferatum* in relation to temperature and water activity. The numbers on the isopleths refer to similar growth rates (mm/day).

3.2. Effect of a_W, temperature and incubation time on fumonisin production

The surface response curves of fumonisin B_1, B_2, B_3 and total fumonisin production at 15, 25, and 30 °C over 28 days of incubation are shown in Figs. 3, 4 and 5. All the abiotic factors under study (a_W, temperature and incubation time) influence fumonisin production by the three *F. proliferatum* strains in wheat based medium. No significant production of either toxin was observed at $a_W \leq 0.92$ for all strains at all temperatures assayed during the incubation period. The maximum amounts of total fumonisins ($FB_1 + FB_2 + FB_3$) were obtained at 25 °C and 0.99 a_W after 21 and 28 days of incubation for *F. proliferatum* ITEM 15664 and ITEM 15661, respectively, while for *F. proliferatum* ITEM 15654 maximum amounts of total fumonisins were obtained at 15 °C and 0.98 a_W after 28 days of incubation. In general, toxin levels produced by *F. proliferatum* ITEM 15664 were higher than those produced by the other strains under almost all temperatures tested.

Two different responses to temperature were observed among all evaluated strains. Total fumonisin production was higher at 25 ºC, decreasing in the following order: 15 and 30 ºC for *F. proliferatum* ITEM 15661 and ITEM 15664, respectively (Figs. 3 and 4). For *F. proliferatum* ITEM 15654 total fumonisin production was higher at

Table 1
Analysis of variance on the effects of water activity (a_W), temperature (T), and different strains (S) and their interactions on growth of *Fusarium proliferatum* on wheat-based media.

Source of variation	df[a]	MS[b]	F[c]
a_w	5	130.57	22954.31*
T	2	61.539	10818.499*
S	2	0.921	161.884*
a_w x T	10	3.932	691.196*
a_w x S	10	0.105	18.372*
T x S	4	0.0942	16.566*
a_w x T x S	20	0.111	19.535*

* $P < 0.001$.
[a] Degrees of freedom.
[b] Mean square.
[c] Snedecor-F.

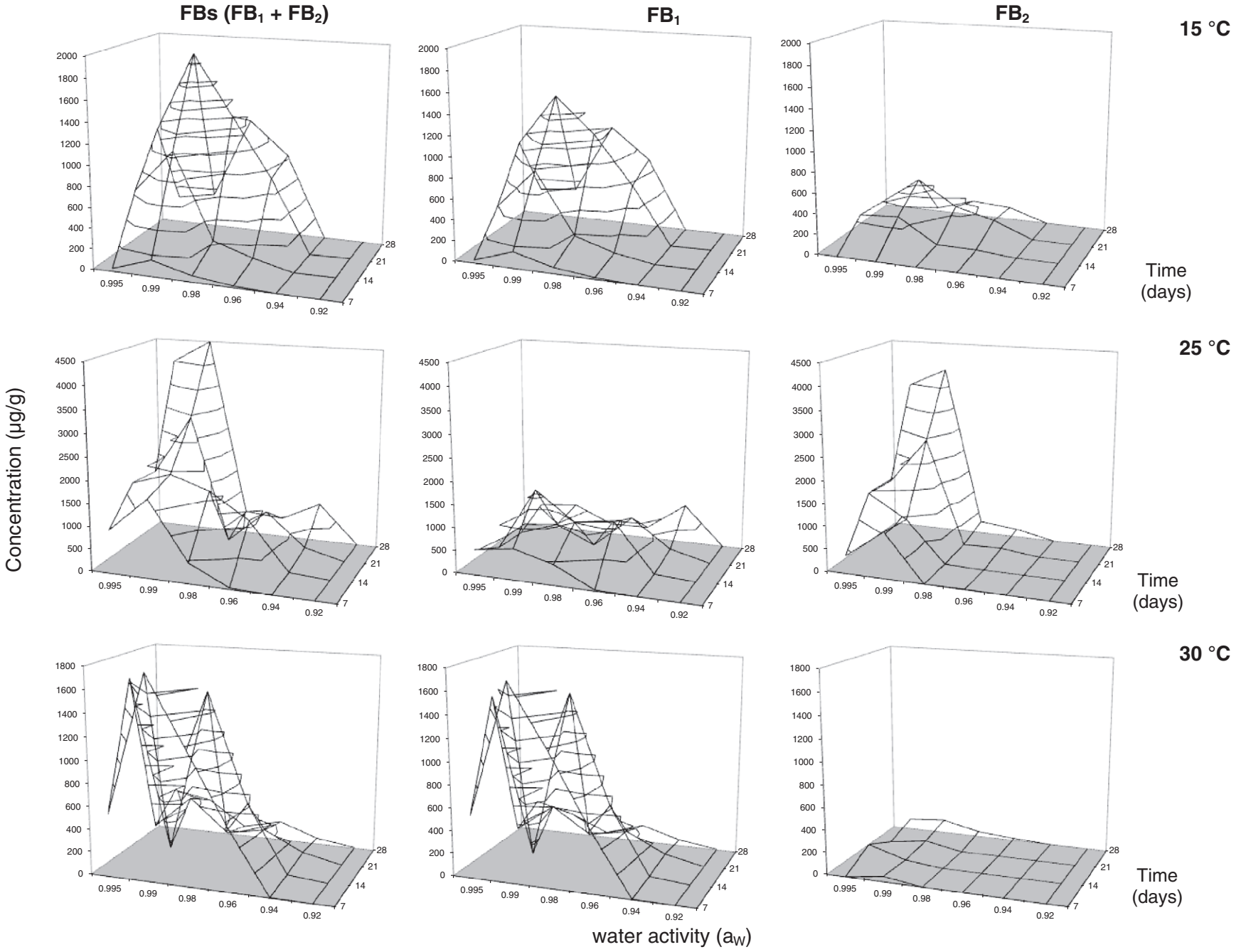

Fig. 3. Fumonisin concentrations (µg/g) produced by *Fusarium proliferatum* ITEM 15661 inoculated onto 2% wheat-base media adjusted to different a_W levels and incubated at 15, 25 and 30 °C for 28 days.

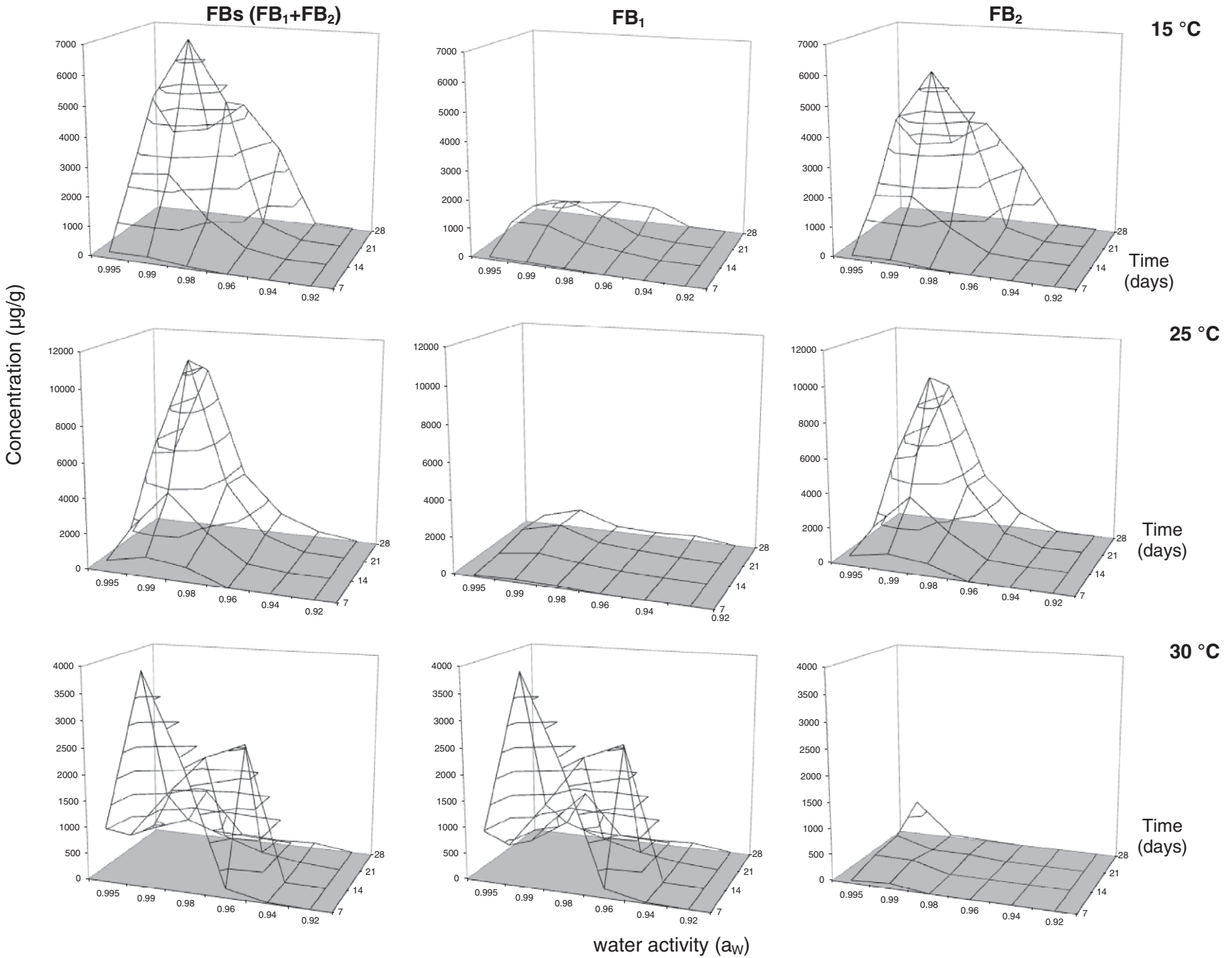

Fig. 4. Fumonisin concentrations (µg/g) produced by *Fusarium proliferatum* ITEM 15664 inoculated onto 2% wheat-base media adjusted to different a_W levels and incubated at 15, 25 and 30 °C for 28 days.

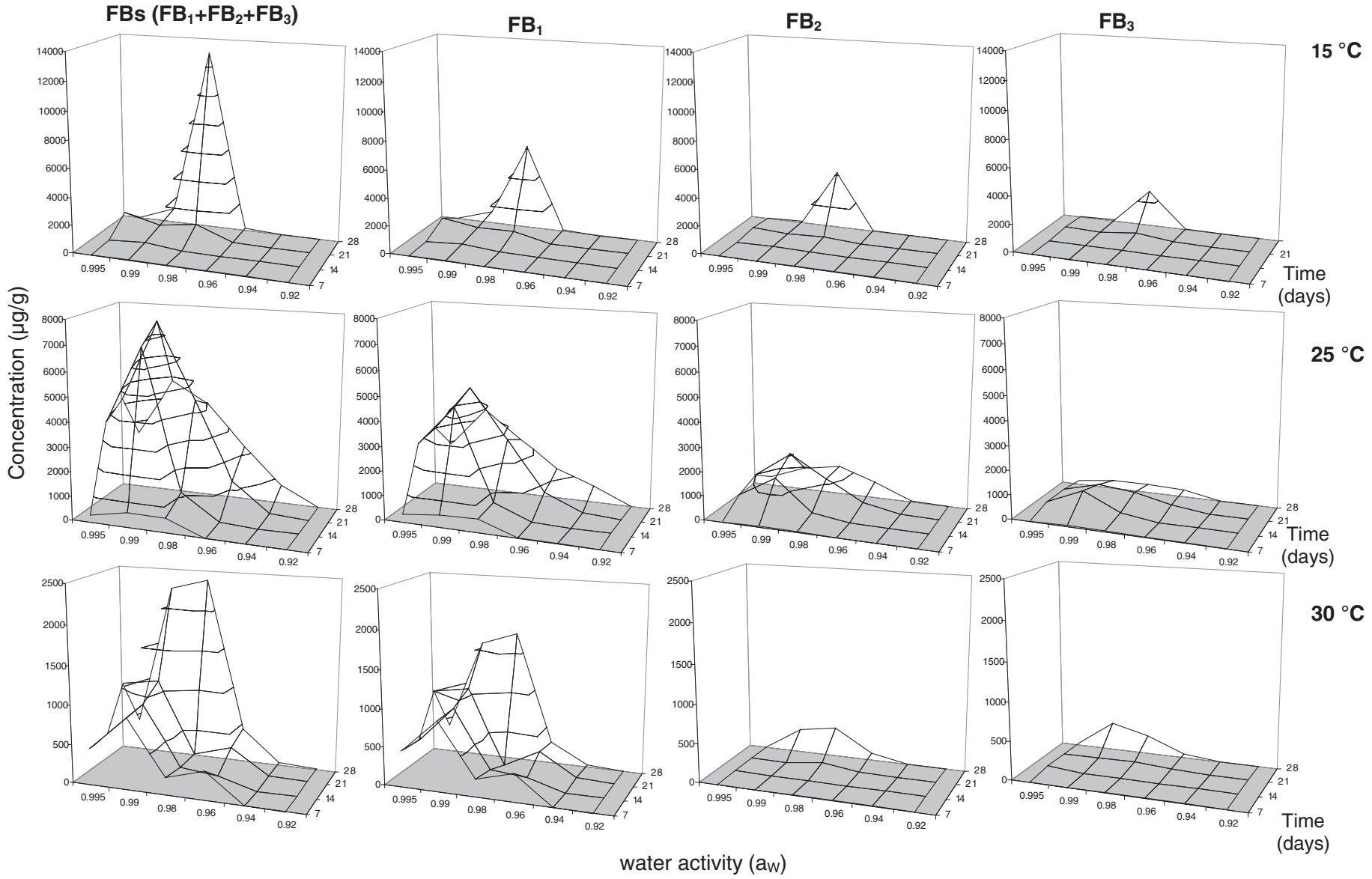

Fig. 5. Fumonisin concentrations (µg/g) produced by *Fusarium proliferatum* ITEM 15654 inoculated onto 2% wheat-base media adjusted to different a_W levels and incubated at 15, 25 and 30 °C for 28 days.

15 °C, decreasing in the following order: 25 and 30 °C, respectively (Fig. 5).

Studied strains had different fumonisin production profiles. *F. proliferatum* ITEM 15661 and ITEM 15664 produced FB_1 and FB_2 (Figs. 3 and 4), but *F. proliferatum* ITEM 15654 was able to produce FB_1, FB_2 and FB_3 (Fig. 5). It is notable that fumonisin production profiles for each particular strain were related to incubation temperatures (Figs. 3, 4 and 5). As an example, for *F. proliferatum* ITEM 15664 most of the fumonisin produced at 25 and 15 °C, around 90%, was FB_2, but at 30 °C the situation was reversed, with FB_1 representing almost the 90% of the total amount of fumonisin produced (Fig. 4).

All single variables (a_W, temperature, and days of incubation) and the two- and three-way interactions significantly influenced total fumonisin production for the three strains (Table 2). Data obtained were used to develop contour maps to identify the optimum conditions of a_W and temperature and the range of conditions for production of different quantities of fumonisins (Fig. 6).

4. Discussion

This study compared for the first time the impact of both a_W and temperature on growth and fumonisin production on wheat-based medium by three strains of *F. proliferatum* isolated from wheat in Argentina during a non-FHB epidemic year. Both variables affected growth, and the pattern obtained was independent of the strains evaluated. Optimal a_W levels for growth ranged from 0.995 to 0.96 at 25–30 °C. No growth was observed at 15 °C at 0.90 a_W, but all strains were able to grow slowly at that a_W level assayed at 25, and 30 °C. Previous studies of the water and temperature requirements for *F. proliferatum* growth reported that this fungus has a minimum of 0.90 a_W, and the temperatures at which growth was possible ranged from 4 to 37 °C in maize extract agar, with optimal growth at the highest a_W tested (0.994) (Marín et al., 2004). Magan and Lacey (1984) found that some *Fusarium* species were able to grow on wheat extract agar at 0.89 to 0.90 a_W and the optimum a_W was 0.995 regardless of temperature, but *F. proliferatum* was not included in that study. Later, Marín et al. (1999) analyzed responses of different *Fusarium* species to a_W and temperature, and found that growth rates of *Fusarium verticillioides* and *F. proliferatum* strains were up to 7 mm/day, and that the trend was very similar for the three different cereal extracts assayed: growth at 5 to 10 °C was slightly faster on wheat extract agar, faster at 25 to 30 °C on barley extract agar, and faster at 37 °C on maize extract agar. These results are similar to those presented above, even though the strains used in the studies cited before were isolated from maize grains.

As *Fusarium* species may be present on a substrate for long periods during which a_W may change, it is important to know both the optimal a_W range for growth and that permitting sub-optimal growth. Under field conditions, temperature fluctuations, changes in relative humidity, and rainfall all influence colonization of developing grain by *F. proliferatum*.

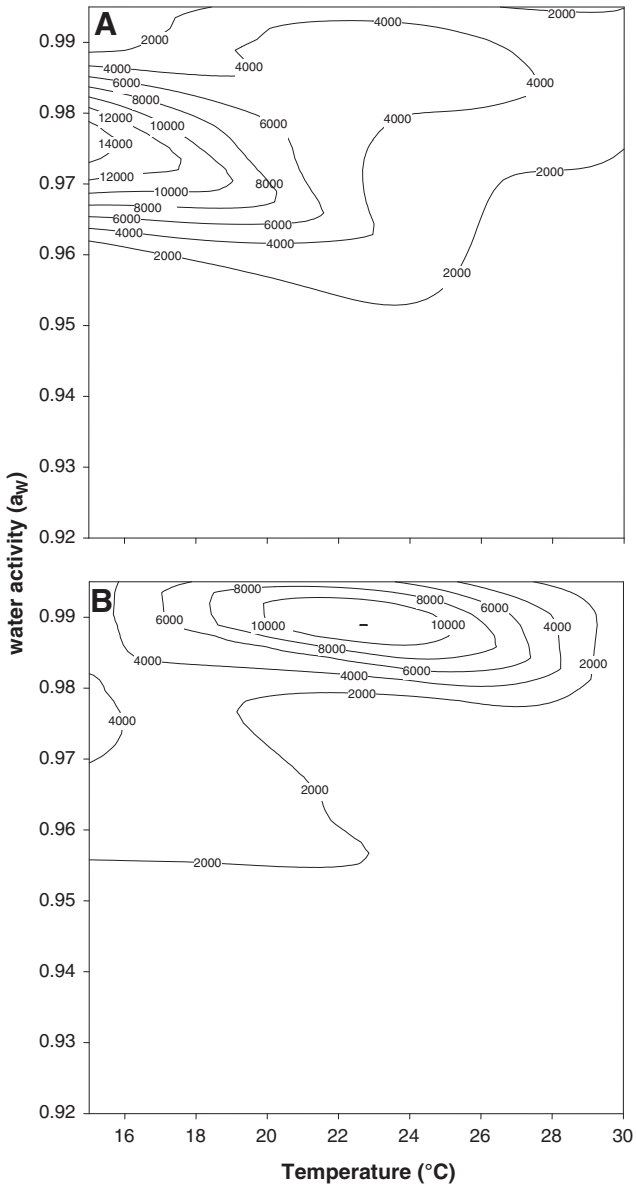

Fig. 6. Two-dimensional contour maps of fumonisin production profiles of *Fusarium proliferatum* (A: ITEM 15654 and B: ITEM 15664) in relation to temperature and water activity. The numbers on the isopleths refer to similar toxin concentrations (μg/g).

In the present study, maximum amounts of total fumonisins were produced at 25 °C and 0.99 a_W after 21 and 28 days of incubation for two of the strains, while for the third, maximum values were at 15 °C

Table 2
Analysis of variance on the effects of water activity (a_W), temperature (T), and incubation time (d) on fumonisin production by three *Fusarium proliferatum* strains on wheat-based media.

Source of variation	ITEM 15661			*Fusarium proliferatum* ITEM 15664			ITEM 15654		
	df[a]	MS[b]	F[c]	df	MS	F	df	MS	F
T	2	64.277	76.120*	2	6.567	15.642*	2	129.349	159.434*
a_W	4	124.081	146.941*	4	132.353	315.263*	4	215.319	265.4*
d	3	18.679	22.120*	3	57.742	137.540*	3	133.686	164.780*
T × a_W	8	10.936	12.951*	8	4.476	10.661*	8	8.000	9.861*
T × d	6	10.914	12.925*	6	29.130	69.388*	6	17.296	21.319*
a_W × d	12	4.213	4.989*	12	6.909	16.456*	12	11.375	14.021*
T × a_W × d	24	3.709	4.392*	24	1.730	4.120*	24	5.215	6.428*

* $P < 0.001$.
[a] Degrees of freedom.
[b] Mean square.
[c] Snedecor-F.

and 0.98 a_W after 28 days of incubation. The limiting a_W for detectable mycotoxin production by *F. proliferatum* was slightly narrower than that for growth. Our results agree with those of Marín et al. (1999) who found that the range of temperatures over which FB_1 may be produced on maize grains is narrower than that under which the fumonisin-producing species (*F. verticillioides* and *F. proliferatum*) are able to germinate and grow.

Different fumonisin (FB_1, FB_2 and FB_3) production profiles were found between the analyzed strains, and those profiles for each particular strain were related to temperature. Medina et al. (2013) studied the relationship between the expression of nine biosynthetic *FUM* cluster genes and growth and FB_1 and FB_2 production in a *F. verticillioides* strain and concluded that temperature and a_W have a profound effect on both gene expression of key biosynthetic genes as well as significantly affecting growth and the phenotypic production of the toxic secondary metabolites. Also in this study optimal temperature for FB_1 and FB_2 production was not the same. The results indicate that the production of different types of fumonisin by *F. proliferatum* is favored by different temperatures. Also it has been established that this species seems better adapted to low temperatures and may produce greater amounts of FB_1 than *F. verticillioides* at these temperatures (Ryu et al., 1999). This is important as wheat is a winter crop and the optimal temperature for cultivation of this cereal ranged between 10 and 24 °C. It is well know that unlike *F. verticillioides*, *F. proliferatum* colonizes a wide range of host plants other than maize, such as wheat and barley among others, with no host preference. A phylogenetic study of a large number of *F. proliferatum* strains from different hosts has revealed a high genetic variability and has also shown that the ability to produce fumonisins was widely distributed indicating that this species can represent a risk for health similar to *F. verticillioides* (Jurado et al., 2010).

Extensive work has been carried out previously on the combined effect of a_W and temperature on fumonisin (mainly FB_1) production on maize-based media and maize grains (Marín et al., 2004). However the work described here provides the first detailed study on fumonisin production as affected by a_W and temperature on wheat-based media. There is one comparable study done by Marín et al. (1999). In this study the authors showed the effect of different substrates (maize, wheat and barley) and a_W levels (0.93, 0.95 and 0.98) on growth and FB_1 production at 25 °C by *F. verticillioides* and *F. proliferatum*. They demonstrated that although fumonisin producers can colonize barley and wheat more rapidly than maize they were unable to produce FB_1 in these cereals. However, there are several points to take into account: firstly the experiment was carried out only at 25 °C which is a suitable temperature for fumonisin production on maize; secondly, the study was limited to FB_1 production and thirdly the isolates used originated from maize. The authors suggest that it is possible that fumonisin could be produced on the other cereals under different temperature regimens.

During the present work the effect of a broad range of a_W (0.995 to 0.90) and temperatures (15, 25 and 30 °C) on FB_1, FB_2, and FB_3 production by 3 strains of *F. proliferatum* isolated from wheat was evaluated. All the conditions evaluated are those which usually occurred during wheat grain development (especially at milk and dough stages) in the field. These results can explain Marín et al. (1999) conclusions about the absence of fumonisin production on wheat at 25 °C. Two out of three strains of *F. proliferatum* produced almost exclusively FB_2 and negligible amounts of FB_1 on wheat based-media at this temperature. Also this kind of profile (more FB_2 than FB_1) has been observed on naturally contaminated wheat grain in Argentina (Palacios et al., 2011). The other strain showed a normal fumonisin profile ($FB_1 > FB_2 > FB_3$) but the production was higher at 15 °C than at the other temperatures evaluated. Also, Marín et al. (1999) study did not include the 0.99 a_W level which was found to be the optimum for fumonisin production in most of the conditions evaluated during the present study.

Thus, field conditions during a non-FHB epidemic year are likely to be conducive to *F. proliferatum* growth and toxin production.

Recently, fumonisin contamination has been reported in both kinds of wheat and sub-products in several countries including Argentina (Busman et al., 2012; Castoria et al., 2005; Cendoya et al., 2014; Chehri et al., 2010; Jakšić et al., 2012; Khosrow et al., 2010; Kushiro et al., 2009; Palacios et al., 2011; Roscoe et al., 2008; Serrano et al., 2012; Wang et al., 2013). Although there is no legislation for fumonisin levels in wheat and wheat products, this legislation does exist for maize, where the maximum limits established for maize and sub-products for human consumption are 1000 ng/g in the European Union and from 2000 to 4000 ng/g in the USA. It is remarkable that some reports of fumonisin contamination of wheat exceed these limits. Due to the importance of wheat in Argentinean population diet and also in many countries around the world, and because it has been proposed, in a study in the Netherlands, that fumonisin intake occurs mainly via the intake of wheat and wheat-products (Bakker et al., 2003), the present results provide useful information for predicting the possible risk factors for fumonisin contamination of wheat. The a_W and temperature ranges evaluated in this study simulate those occurring during wheat grain development (especially milk and dough stages) in the field. The data indicate the contrasting impact of a_W, temperature, and incubation time on growth and production of FB_1, FB_2 and FB_3 by the three *F. proliferatum* strains examined. The most important finding is that fumonisin production profiles for each strain were related to incubation temperatures.

Since *F. proliferatum* is isolated at a very high frequency from wheat around the world the contour maps of growth and fumonisin production generated by the present study may provide very useful guidelines for facilitating effective management of predicting risk for growth and mycotoxin production during ripening, harvesting and storage of wheat.

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