Research Article

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Fusarium species and moniliformin occurrence in sorghum grains used as ingredient for animal feed in Argentina

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Abstract

BACKGROUND: A survey on *Fusarium* species and moniliformin (MON) occurrence in sorghum grains collected from one of the main sorghum-producing areas of Argentina was conducted. Also, growth of *F. thapsinum*, one of the main sorghum pathogens, and MON production under different water activity (a_w) conditions on a sorghum-based medium were determined.

RESULTS: Infection of sorghum grains by *Fusarium* species ranged from 82.5 to 99%; closely related species *F. verticillioides*, *F. thapsinum* and *F. andiyazi* were the most frequently recovered, followed by *F. proliferatum* and *F. subglutinans*. By sequencing a portion of the translation elongation factor-1α (TEF-1α) gene and by maximum parsimony analysis, *F. verticillioides* and closely related species were identified as *F. thapsinum*, *F. andiyazi* and *F. verticillioides*. Species within the *F. graminearum* species complex (FGSC) were isolated in high frequency. Maximum growth rates of 12 *F. thapsinum* strains were obtained at 0.995 a_w. All evaluated strains were able to produce MON at all a_w values tested, but MON production was higher at 0.995–0.982 a_w. MON was detected in 41% of the samples at levels ranging from 363.2 to 914.2 μg kg^{-1}.

CONCLUSION: This study provides new data on the occurrence of *Fusarium* species in sorghum grains destined for animal consumption in Argentina. The production of MON at different a_w values showed that the toxin can be produced under field conditions. The risk to livestock exposed to daily low levels of MON associated with the toxin occurrence in the sorghum grains analyzed is unknown.

Keywords: sorghum grains; *Fusarium* species biodiversity; *Fusarium thapsinum*; growth rate; moniliformin

INTRODUCTION

Sorghum (*Sorghum bicolor* L) is globally the fifth most important grain crop after wheat, rice, maize and barley. Argentina ranks second (along with Australia) after the USA in global sorghum exports, with 37% of the annual production being shipped to Mexico, Japan and Colombia. Of the average 5 million tons produced annually, 0.6–1.2 million tons are currently exported, but the largest volume is used for local consumption.[1] In Argentina, sorghum is a summer crop and its production is concentrated in Santa Fe and Córdoba provinces (Pampas region), an area with optimal conditions for this crop. Sorghum grain is used as livestock feed for beef and dairy cattle and also for bioethanol production.[2] Moreover, the sorghum crop is used as an essential part of a rotation system that maintains productivity and soil structural stability.

Several fungal diseases are relevant for sorghum, including grain mold, anthracnose, stalk rot, ergot, smuts and downy mildew.[3] Disease severity depends upon the consortium of species present and the environmental conditions during the growing season. Grain mold and stalk rot of sorghum are both yield-limiting diseases and reduce seed quality and vigor.[4] Losses typically result from poor grain fill, lodged peduncles and/or stalk breakage that cause problems during harvest, and these conditions generally occur after anthesis.[5] *Fusarium* species usually associated with sorghum belong to the *Fusarium fujikuroi* species complex (FFSC). Some species can be mycotoxin producers; these metabolites are harmful to both humans and animals that consume contaminated grains.[6] The FFSC includes more than 40 phylogenetic species, which are heterothallic fungi requiring opposite mating types (*MAT-1* and *MAT-2*) for sexual crosses to occur. Twelve mating populations within the FFSC have been described (MP A–L).[7,8]

Fusarium stalk rot and grain mold are the most common diseases in all sorghum-growing regions of the world, and *F. thapsinum* is the main pathogen causing both diseases.[9–13] Another member within the FFSC, *F. andiyazi*, has been recovered from most sorghum-growing regions of the world, including South America, South Africa, Australia, Ethiopia, Nigeria and the USA.[7,14] *Fusarium andiyazi* is pathogenic to sorghum seedlings in *in vitro* assays and is associated with stalk rot.[11,12,15,16] *Fusarium* species produce a wide variety of mycotoxins; their production is influenced by environmental conditions, crop management and storage methods. Natural occurrence of *Fusarium* toxins in different crops includes trichothecenes (T-2/HT-2 toxin, deoxynivalenol,

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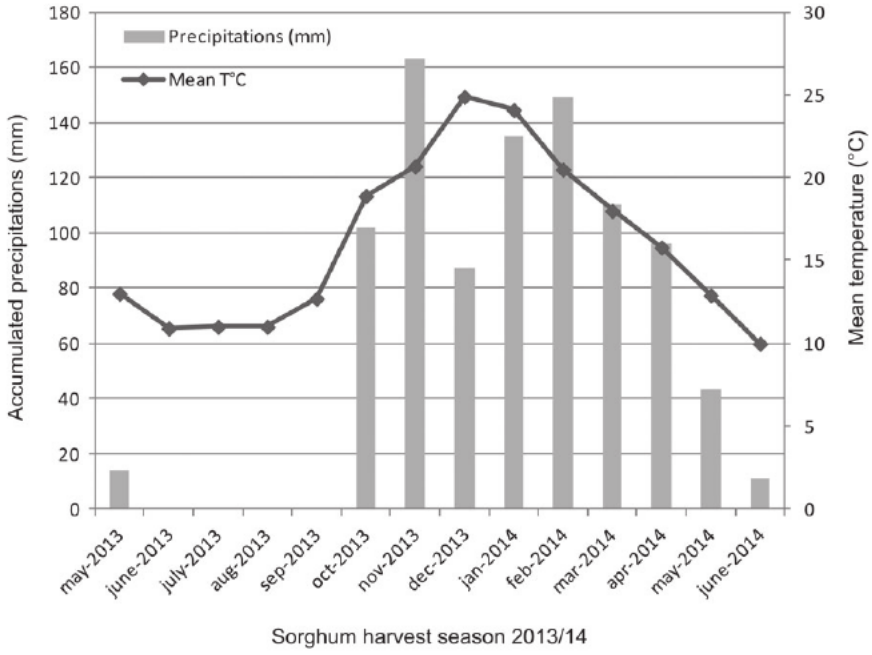

Figure 1. Mean temperature and accumulated precipitations during sorghum-growing season 2013/14.

diacetoxyscirpenol, nivalenol), zearalenone, fumonisins and moniliformin (MON).[6] MON is a sodium or potassium salt of 1-hydroxycyclobut-1-ene-3,4-dione that is capable of causing disease in domestic animals, possibly through selective inhibition of mitochondrial pyruvate and α-ketoglutarate oxidations, preventing the entrance of pyruvate and α-ketoglutarate into the tricarboxylic acid cycle and thus reducing oxidative phosphorylation, i.e. adenosine triphosphate (ATP) production.[17,18] Clinical signs of MON toxicity observed in animals have been generally described as progressive muscular weakness, respiratory distress, cyanosis and coma followed by death.[19] The species that are known to produce MON are *F. proliferatum*, *F. avenaceum*, *F. fujikuroi*, *F. nygamai*, *F. pseudonygamai*, *F. subglutinans*, *F. verticillioides* and *F. thapsinum*, among others.[20]

Although some mycological surveys of sorghum grains have been carried out, there is scarce information worldwide, including in Argentina, on the occurrence of *Fusarium* species and MON in sorghum grains destined for animal consumption. Therefore the aims of this study were (i) to evaluate the biodiversity of *Fusarium* species from sorghum grain samples collected from Córdoba province, Argentina during the 2013/14 harvest season, (ii) to determine the occurrence of MON in sorghum grains and (iii) to evaluate growth rate and MON production under different water activity conditions on a sorghum-based medium by *F. thapsinum*, one of the main sorghum pathogens.

MATERIALS AND METHODS

Sorghum sampling

Forty-eight 2.5 kg sorghum samples were collected from two fields (distant 50 km). Twenty-four samples from each field were taken following a polar sampling design during the harvest stage in the south of Córdoba province, harvest season 2013/14.[7] The crop rotation system was soybean/sorghum and the sorghum hybrids sown were VDH422 and DK61T, both of high tannin content. The climatic conditions in these fields (supplied by the Meteorological Service of UNRC) during the sorghum-growing season are shown in Fig. 1. Samples were immediately analyzed for fungal contamination. Then they were dried in a forced draft oven at 60 °C for 48 h and stored at 4 °C until mycotoxin analyses.

Fusarium isolation and identification

From each sorghum sample, 100 grains were plated (ten grains per Petri dish) on modified pentachloronitrobenzene (PCNB) medium[7] after grain surface disinfection. Briefly, 100 grains of each sample were immersed in 0.4% chlorine for 2 min and rinsed twice with sterile distilled water for 1 min.[21] The PCNB plates were incubated at 25/20 °C under 12/12 h photoperiod light (cold white and black fluorescent lamps)/darkness respectively for 7 days. The percentage of infection of sorghum grains by *Fusarium* species was determined and these species were identified according to Leslie and Summerell.[7] Briefly, 50 isolates from each sample were taken and subcultured on Spezieller Nahrstoffarmer agar (SNA) plates for 5–7 days at 25/20 °C under 12/12 h photoperiod light (cold white and black fluorescent lamps)/darkness respectively. After that, morphological identification of isolates (representatively selected from different samples) was done by single-spore culturing on carnation leaf agar (CLA) and potato dextrose agar (PDA) plates. Plates were incubated for 10–14 days at 25/20 °C under 12/12 h photoperiod light (cold white and black fluorescent lamps)/darkness respectively. Macromorphology of fungal colonies such as mycelium color and agar pigmentation was observed on PDA, and micromorphology including conidium, conidiophore, chlamydospore, pseudochlamydospore and sporodochium production was observed on CLA. The isolated strains were kept cryopreserved in sterile 15% glycerol[7] and maintained in the culture collection at the Department of Microbiology and Immunology, UNRC.

Species identification by sequencing translation elongation-1α gene

Thirty-six strains morphologically identified as *F. verticillioides* and closely related species representative from the different

samples analyzed were identified by sequencing a portion of the translation elongation factor-1α (TEF-1α) gene. The strains were grown in complete medium[7] and incubated on an orbital shaker (150 rpm) for at least 3 days at 25 ± 1 °C. The resulting mycelia were harvested by filtration through non-gauze milk filters (Ken AG, Ashland, OH, USA). Excess water was removed by blotting mycelia between clean paper towels, and dried mycelia were stored frozen at −20 °C. DNA extraction was performed by the cetyltrimethylammonium bromide (CTAB) method.[7] Analysis of the partial sequence of TEF-1α was made following polymerase chain reaction (PCR) amplification with primers described by O'Donnell *et al.*[22] The PCRs were conducted with 10–20 ng of fungal DNA in a total volume of 50 µL of 1× reaction buffer containing 1.5 mmol L^{-1} $MgCl_2$, 0.5 U of Taq DNA polymerase (5 U μL^{-1}; Invitrogen Life Technologies, Buenos Aires, Argentina), 0.2 mmol L^{-1} dNTPs and 0.3 µmol L^{-1} of each TEF-1α primer (EF1 5′-ATGGGTAAGGAGGACAAGAC-3′, EF2 5′-GGAAGTACCAGTGATCATGTT-3′). A negative control, containing all reagents but no fungal DNA, was included in every set of reactions. PCR was conducted in a PTC-200 Thermal Cycler (MJ Research Inc., Watertown, MA, USA) according to the following cyclic conditions: initial denaturation at 94 °C for 1 min, followed by 35 cycles consisting of 94 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min, and a final extension step of 72 °C for 5 min, and then held at 4 °C indefinitely. The TEF-1α amplification was verified by agarose (1.5%) gel electrophoresis of 5 µL of each reaction mixture with 1× Tris-acetic acid EDTA buffer (pH 8.0). TEF-1α amplicons were sequenced bi-directionally (Macrogen Inc., Seoul, Korea). Each forward strand was pairwise aligned to its respective reverse complement using BioEdit Version 7.0.9.0,[23] and then sequencing strain errors were revised. Nucleotide sequence comparisons were performed using the Basic Local Alignment Search Tool (BLAST) network services of the National Centre for Biotechnology Information (NCBI) and the Fusarium Identification Database (Fusarium ID, http://isolate.fusariumdb.org/) with TEF-1α sequences as the query. Multiple sequence alignment of the TEF-1α sequences was performed using the web-based program MAFFT.[24] The partial TEF-1α sequences from reference strains of the FFSC were included in the analysis. Based on this alignment, maximum parsimony analysis was performed with a heuristic search with random addition sequences, branch swapping by tree bisection–reconnection (TBR) and MAXTREES set at 10000, using TNT Version 1.1.[25] Relative robustness of the individual branches was estimated by bootstrapping using 10 000 replicates. *Fusarium oxysporum* strain NRRL22902 (GenBank accession AF1603212.1) was used as outgroup.

Fusarium thapsinum growth and moniliformin production

Fungal strains

Twelve *F. thapsinum* strains isolated from the sorghum grain samples identified by morphological and phylogenetic analysis were evaluated to determine the influence of water activity on growth, lag phase and MON production. The strains were kept in the culture collection at the Department of Microbiology and Inmunology, UNRC.

Medium

Sorghum grains free of MON contamination were ground in a Romer mill (range 60–200 mesh; Romer Labs Inc., Union, MO, USA). Mixtures of 20 g L^{-1} milled sorghum grains in water were prepared with 20 g L^{-1} Agar Technical (Agar No. 3, Oxoid Ltd, Basingstoke, UK). The water activity (a_w) of the sorghum-based medium was adjusted to 0.995, 0.982 and 0.955 by addition of different amounts of glycerol.[26] The media were autoclaved at 120 °C for 20 min. Flasks of molten media were thoroughly shaken prior to pouring the media into 9 cm sterile Petri dishes. The water activity of the media was checked at 0.995, 0.982 and 0.955 a_w using an AquaLab Series 3 meter (Decagon Devices, Inc., Pullman, WA, USA). Additional, uninoculated control plates were prepared and the a_w was measured at the end of the experiment in order to detect any significant deviation.

Inoculation, incubation and growth measurement

Petri plates containing sorghum-based medium at different a_w conditions were inoculated (in triplicate) with a 5 mm diameter agar disk taken from the margin of a 7-day-old colony of each isolate grown on SNA at 25 °C. Inoculated plates of the same a_w were sealed in polyethylene bags and incubated at 25 °C for 21 days. 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. Radii of the colonies were plotted against time, and linear regression was applied in order to obtain the growth rate (mm day^{-1}) as the slope of the line. After the incubation period, uninoculated controls and treatments were evaluated for MON concentrations.

Moniliformin production

MON extraction was done from the culture media (~20 g) from the treatment and control plates with 50 mL of acetonitrile/water (84:16 v/v) following the methodology proposed by Parich *et al.*[27] Briefly, samples were shaken for 30 min in an orbital shaker (150 rpm) and filtered through Whatman No. 1 filter paper. A volume of 20 mL of each extract was dried on a rotary evaporator (Büchi, Valinhos, SP, Brazil). Meanwhile, a SAX solid phase extraction (SPE) column (Bond Elut SAX, 500 mg, 6 mL; Agilent, Santa Clara, CA, USA) was conditioned by adding 2 mL of methanol followed by 2 mL of water and 2 mL of 0.1 mol L^{-1} phosphoric acid. The evaporated extract was dissolved in 5 mL of methanol (high-performance liquid chromatography (HPLC) grade), shaken for 1 min and transferred onto the conditioned SAX SPE column. After the extract had passed through the column, it was washed with 2 mL of 0.1 mol L^{-1} phosphoric acid and 2 mL of water and air dried for 15 min. Finally, the toxin was eluted as MON–tetrabutylammonium hydroxide ion pairs. An ion pair solution was prepared by mixing 50 mL of 20% tetrabutylammonium hydroxide and 100 mL of 1.1 mol L^{-1} potassium dihydrogen orthophosphate and the pH was adjusted to 7.0. Then an elution solution was prepared by diluting the ion pair solution with water (1:1 v/v). Finally, the toxin was eluted with 2 mL of elution solution. Before analyzing the sample by HPLC, the eluted extract was passed through a 0.45 µm filter.

The HPLC system consisted of a Hewlett-Packard 1100 pump (Palo Alto, CA, USA) connected to a UV detector and a Hewlett-Packard Kayak XA data module (HP Chem Station Rev. A.06.01). The column used was a 150 mm, 4.6 mm i.d., 5 µm Luna-Phenomenex C18(2) column with a 4 mm, 3 mm i.d. Security Guard cartridge of the same material (Phenomenex, Torrance, CA, USA). The mobile phase was a water/acetonitrile/ion pair solution (94:5:1 v/v/v) using an isocratic method at a flow rate of 1 mL min^{-1}. Detection was made at 228 nm and the retention time of MON (checked by the co-injection technique) was 14 min.

MON quantification was made as peak areas and compared with reference standard solutions (Sigma Chemical Co., St Louis, MO, USA). Standard solutions of 25, 12.5, 6.25, 3 and 1 µg mL^{-1} in the mobile phase were prepared from a 1 mg mL^{-1} solution of pure MON (Sigma-Aldrich Co.) in acetonitrile after solvent evaporation. A recovery experiment was performed in triplicate by spiking plates of MON-free sorghum-based medium (10 g) with MON at levels of 12.5, 25 and 50 mg kg^{-1}. Spiked samples were left overnight at room temperature to allow solvent evaporation prior to proceeding with the extraction step. Mean recovery was 76%. The limit of detection (LOD) of the analytical method determined at a ratio S/N = 3 was 23 µg kg^{-1}, where S denotes signal (intensity of the toxin peak) and N denotes signal noise. Relative standard deviations (RSDs) for both intra- and inter-day assays were computed to verify repeatability and reproducibility respectively. The RSD for repeatability was 0.79% and for reproducibility was 2.86%.

Statistical analysis

The statistical effects of a_w on growth rate, lag phase and MON production were evaluated by analysis of variance (ANOVA) using InfoStat for Windows 2012 Version 2.03 (SPSS Inc., Córdoba, Argentina). Statistical significance was judged at the level $P \leq 0.0001$. Then Fisher's least significant difference (LSD) test was used for determination of the significance of each a_w level on lag phase, growth rate and MON production by the strains (significance judged at $P \leq 0.05$).

Moniliformin natural occurrence in sorghum grains

Samples (1 kg) were ground in a Romer mill (range 60–200 mesh; Romer Labs Inc.) and thoroughly mixed. Ground subsamples (25 g) were shaken in 100 mL of acetonitrile/water (84:16 v/v) for 30 min and filtered through Whatman No. 1 filter paper. A 5 mL extract was transferred to the glass tube supplied with a MycoSep 240-Mon column extraction kit (Romer Labs Inc.), and extract cleaning was performed following the instructions of the manufacturer. Then 2 mL of the purified extract was collected and evaporated to dryness under N_2 flow. MON was quantified by injecting 50 µL of the extract of each sample dissolved in mobile phase into the HPLC system. Before analyzing the sample by HPLC, the eluted extract was passed through a 0.45 µm filter. The mobile phase and standard solutions used were as described above. The LOD (S/N = 3) determined in sample extracts and standard solutions of MON was 0.22 mg kg^{-1}.

RESULTS

Fusarium species in sorghum grains

The percentage of infection of sorghum grain samples by *Fusarium* species ranged from 82.5 to 99%. From 688 *Fusarium* strains isolated, a subgroup of 203 strains selected representatively was identified at morphological species level. Within the FFSC, *F. verticillioides* and closely related species were the most frequently recovered, followed by *F. proliferatum* and *F. subglutinans* (Table 1). Some strains identified as *F. verticillioides* and closely related species showed yellow pigmentation on PDA medium, but most of them showed violet, cream yellow or cream pigmentation on PDA. Species within the *Fusarium graminearum* species complex (FGSC) were also isolated in high frequency (26.3%). Other *Fusarium* species identified at morphological species level were those included in the *Fusarium incarnatum-Fusarium equiseti* species complex (FIESC; such as *F. semitectum*) and the *Fusarium oxysporum* species complex (FOSC) and *F. sporotrichioides* (Table 1).

Table 1. Isolation frequency of *Fusarium* species from two sorghum fields sampled in Córdoba province during harvest season 2013/14

Fusarium species from sorghum (morphological species)	Field 1	Field 2
F. verticillioides[a]	37.5	48.3
F. proliferatum	12.5	19.5
F. subglutinans	7.5	8.5
FOSC	ND	0.9
FSSC	6.3	2.5
FIESC (*F. semitectum*)	1.3	0.9
FGSC	35.0	18.6
F. sporotrichioides	ND	0.9

FOSC, *F. oxysporum* species complex; FSSC, *F. solani* species complex; FIESC, *F. incarnatum-F. equiseti* species complex (*F. semitectum* belongs to the FIESC owing to uncertainty in the taxonomy of the group[28]); FGSC, *F. graminearum* species complex; ND, not detected.
[a] Identified as *F. thapsinum*, *F. andiyazi* and *F. verticillioides* after sequencing of translation elongation factor-1α (TEF-1α) gene.

The identification by sequencing a portion of the TEF-1α gene showed that strains identified morphologically as *F. verticillioides* and closely related species were mainly *F. thapsinum*, *F. andiyazi* and *F. verticillioides*. Sequence analysis by the BLAST tool against other sequences from reference strains revealed that 12 strains had high match with published *F. thapsinum* TEF-1α sequences in GenBank and Fusarium ID (100% identity), 12 strains with *F. andiyazi* (99–100% identity), 11 strains with *F. verticillioides* (100% identity) and one strain with *F. proliferatum* (100% identity) (February 2018, http://blast.ncbi.nlm.nih.gov/Blast.cgi), supporting their identification as *F. thapsinum*, *F. andiyazi*, *F. verticillioides* and *F. proliferatum* respectively. The obtained TEF-1α sequences were deposited in GenBank under accession numbers MG550921–MG550956 (http://www.ncbi.nlm.nih.gov/nucleotide). A phylogenetic analysis performed using the sequences obtained together with sequences of other *Fusarium* species belonging to the FFSC retrieved from the Fusarium ID and GenBank databases allowed confirmation of the BLAST results, since the sequenced strains were clustered with the corresponding references strains supported by high bootstrap values (99–100%) (Fig. 2).

Members of the FFSC such as *F. thapsinum*, *F. andiyazi* and *F. verticillioides* were more frequently isolated from field 2, while the species within the FGSC were isolated at higher frequency from field 1 (Table 1).

Fusarium thapsinum growth and moniliformin production

The ANOVA test revealed a statistically significant effect of water activity on growth rate, lag phase and MON production by the *F. thapsinum* strains ($P \leq 0.0001$) (Table 2).

The three a_w levels showed a significant effect on growth rate and lag phase, whereas 0.995 and 0.982 a_w did not show differences in relation to MON accumulation by the strains evaluated ($P \leq 0.05$) (Figs 3 and 4). The growth rate was maximum at 0.995 a_w (5.18–5.85 mm day^{-1}) and decreased as a_w was reduced ($P \leq 0.05$) (Fig. 3). The lag phase increased significantly as a_w was reduced (1.30–2.17 days, $P \leq 0.05$) (data not shown). All assayed strains

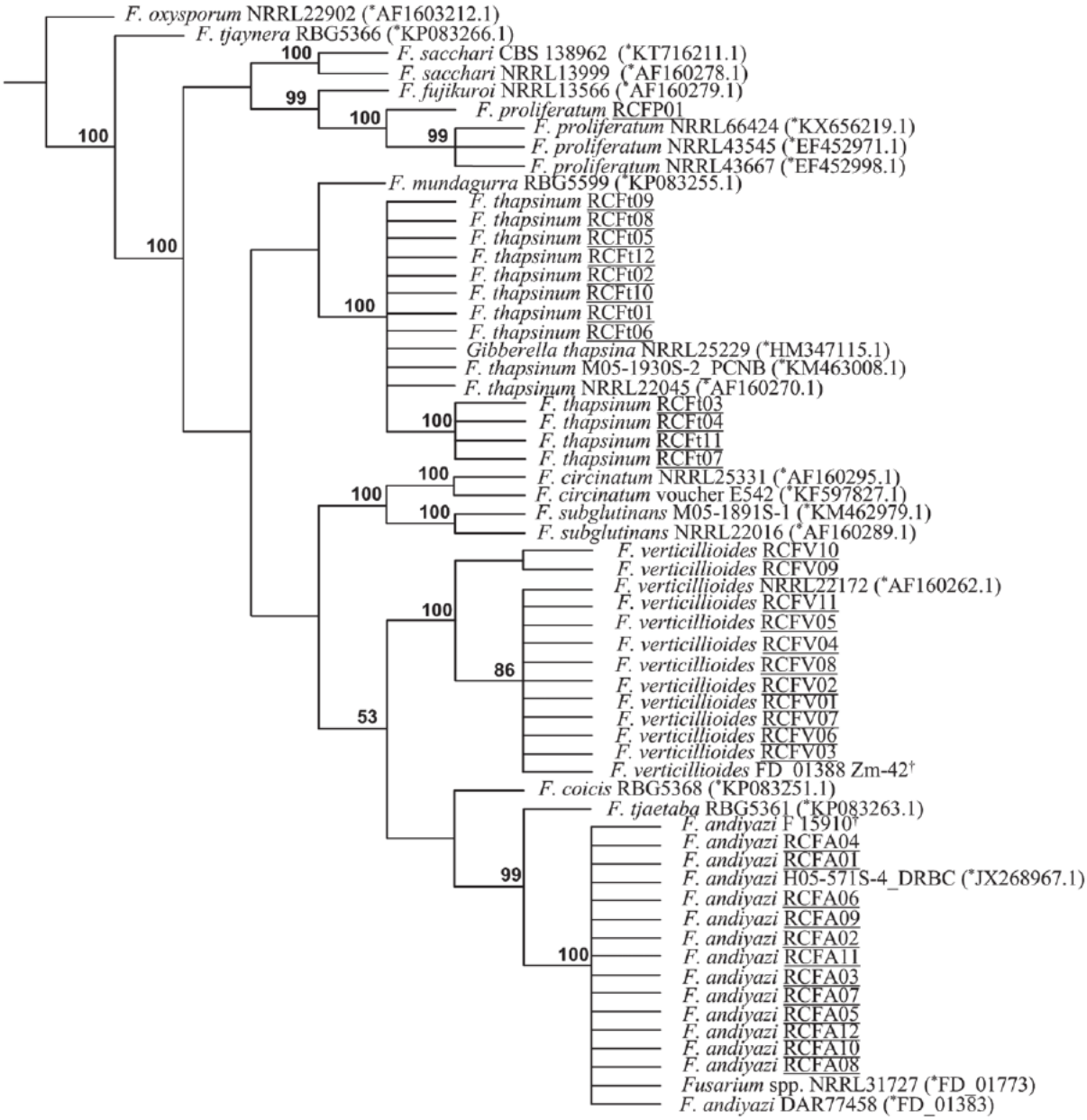

Figure 2. Maximum parsimony tree inferred from translation elongation factor-1α gene (TEF-1α) sequences of species belonging to *Fusarium fujikuroi* species complex (FFSC). Bootstrap values higher than 50% are shown above the branches. *Fusarium oxysporum* strain NRRL22902 was used as outgroup. The sequenced strains from this study are shown underlined. IC, 0.68; IR, 0.93.*GenBank accession numbers. † Fusarium ID accession numbers of TEF-1α sequences not deposited in GenBank.

Table 2. ANOVA on effect of water activity (a_w) on growth, lag phase and moniliformin (MON) production by *Fusarium thapsinum* strains on sorghum-based medium

Item	Growth rate	Lag phase	MON
SS	156.28	3.8	0.00059
DF	2	2	2
MS	78.14	1.9	0.00003
F	3079.62	420.35	10.01
P	<0.0001[a]	<0.0001[a]	0.0001[a]

SS, sum of squares; DF, degrees of freedom; MS, mean square.
[a] The factor had a significant effect ($P \leq 0.0001$) according to the ANOVA test.

were able to produce MON in the range of a_w tested. MON production was significantly influenced by the water activity. In general, MON production by *F. thapsinum* was highest at 0.995–0.982 a_w. The highest MON level was produced by the strain RCFt08 at 0.982 a_w (2118.1 µg kg^{-1}), while the optimal a_w for MON production for the remaining assayed strains was 0.995 (Fig. 4).

Natural occurrence of moniliformin

Natural occurrence of MON was observed in sorghum grain samples collected from both fields evaluated. A higher percentage of contamination was observed in samples from field 2, although the mean MON levels found in the two fields were similar; MON was detected in 41% of the analyzed samples at levels ranging from 363.2 to 914.2 µg kg^{-1} (mean value 605.06 µg kg^{-1}) (Table 3).

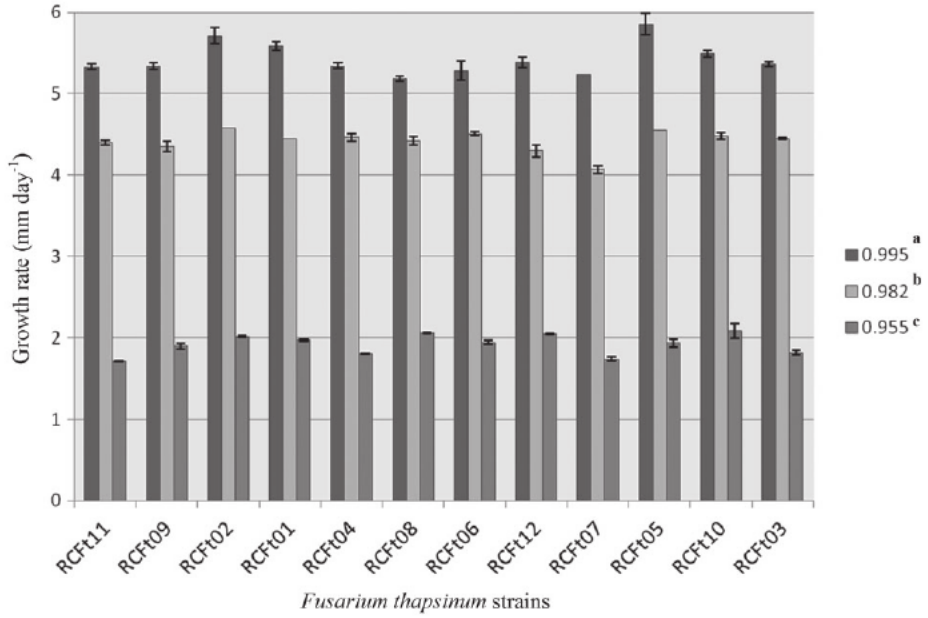

Figure 3. Growth rates produced by *Fusarium thapsinum* strains grown on sorghum-based medium at different a_w levels and 25 °C during 14 days.

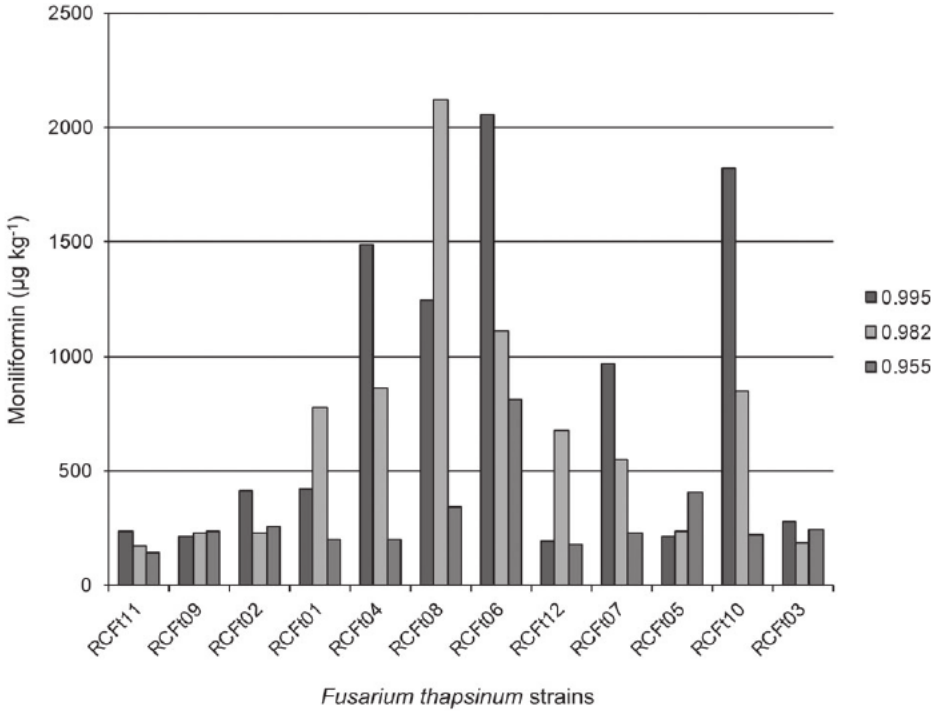

Figure 4. Moniliformin levels produced by *Fusarium thapsinum* strains grown on sorghum-based medium at different a_w levels and 25 °C during 14 days. Results were not corrected by recovery.

DISCUSSION

In the present study, a survey of *Fusarium* species and MON occurrence in sorghum grain samples collected from a main sorghum-producing region of Argentina was conducted. The percentage of infection of sorghum grains with *Fusarium* species was high; the main species isolated belonged to the FFSC, including *F. thapsinum*, *F. andiyazi* and *F. verticillioides*. These results are similar to those obtained by Petrovic *et al.*[11] and Kelly *et al.*,[29] who carried out mycological surveys on sorghum grains in Australia and reported that *F. thapsinum* and *F. andiyazi* were the most frequently isolated species. In the present study, strains of *F. thapsinum* produced, in general, violet, cream yellow or cream pigmentation on PDA. Therefore *F. thapsinum* strains that do not produce the characteristic yellow pigment are morphologically indistinguishable from *F. verticillioides* and *F. andiyazi*, even though *F. andiyazi* can be distinguished from *F. thapsinum* by the production of pseudochlamydospores. Accurate identification of these species requires the use of molecular tools such TEF-1α sequence data. Because different *Fusarium* species possess variable mycotoxigenic potential, accurate identification is necessary.

Table 3. Moniliformin natural occurrence in sorghum grain samples

Sorghum sample	Positive samples/total	Moniliformin (μg kg^{-1})[a] Range (min–max)	Mean value
Field 1	6/20 (30%)	363.2–815.0	549.1
Field 2	10/19 (52.6%)	389.5–914.2	596.7

[a] Detection limit (LOD, S/N = 3) 220 μg kg^{-1}.

Fusarium verticillioides produces large amounts of fumonisins but low levels of MON, whereas *F. thapsinum* produces low levels of fumonisins but high levels of MON and fusaric acid, and *F. andiyazi* produces low levels of fumonisins and MON.

Moreover, species within the FGSC were isolated in high frequency; this can be explained by the soybean–sorghum crop rotation system in the evaluated fields. Species within the FGSC can remain in the soybean stubble and infect the sorghum grain in the next season. *Fusarium graminearum* is frequently isolated from soybean crops in Argentina.[30,31] Burgess *et al.*[32] proposed that sorghum only acts as an asymptomatic host of *F. graminearum*, but this condition can be a toxicological risk, since this species has the potential to produce deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), nivalenol (NIV) and zearalenone (ZEA).[33] Navi *et al.*[34] and Balota[35] showed that the infection of sorghum by species within the FGSC caused reduction of kernel germination, mass, density and nutritional and storage quality of the grains.

This study provides new data on the occurrence of *Fusarium* species in sorghum grains destined for animal consumption in Argentina. Also, the effect of water activity on MON production showed that the toxin can be produced under field conditions. Variation in MON production ability by different strains of *F. thapsinum* in the *in vitro* study was observed; this pattern is similar to other species reported to produce this mycotoxin.[20] During the 2013/14 growing season, the accumulated precipitations were higher than during previous years. These conditions could be favorable for fungal colonization of the sorghum grains and MON production by *F. thapsinum*, since the evaluated strains showed maximum growth rates and MON production at the highest water activities assayed, i.e. 0.982–0.995 a_w. These water activities are normally found in sorghum grains during development stages under field conditions. The MON levels detected in the present study are lower than those detected in maize and wheat in other countries.[36] Although there is no information on natural mycotoxicosis in animals or humans related to MON consumption, toxicity of this mycotoxin has been observed in different animal species, including poultry, rats, mice and mink, among others.[36] The risk to livestock exposed to daily low levels of MON associated with the toxin occurrence in the sorghum grains analyzed is unknown, as information on the toxicity or absorption, distribution, metabolism and excretion (ADME) of the toxin after oral administration is scarce.[19]

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