JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fungal and Fumonisins Contamination in Argentine Maize (*Zea mays* L.) Silo Bags

ANA M. PACIN,[†,‡,§] EMILIA CIANCIO BOVIER,[†] HÉCTOR H. L. GONZÁLEZ,[||,⊥] ELENA M. WHITECHURCH,*[,†,||] ELENA J. MARTÍNEZ,[#] AND SILVIA L. RESNIK[‡,▽]

Fundación de Investigaciones Científicas Teresa Benedicta de la Cruz, M. Dorronzoro 141, Luján B6700FTA, Buenos Aires, Argentina, Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina, Universidad Nacional de Luján, Luján, Argentina, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina, Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad de Buenos Aires, Argentina, Instituto de Cálculo. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina, and Departamento de Industrias y Química Orgánica. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

Fumonisins in maize (*Zea mays* L.) grain silo bags in the conditions of three Argentine provinces were analyzed to determine how this kind of storage affects contamination and if differential storage durations or times of the year of silo bag closing and opening are factors that could modify it. Moisture content, water activity (a_w), molds and yeasts present, and fumonisins were analyzed in 163 maize silo bags, at the moment of closing and later at opening. Storage durations ranged from 120 to 226 days. The analysis was centered on fumonisins since most samples were only contaminated with these toxins. Fumonisins, moisture content, and a_w increased significantly, whereas mold propagules/g and yeasts CFU/g did not present significant differences between silo opening and closing. The date on which silo bags were closed and later opened, however, did affect the level of fumonisin contamination.

KEYWORDS: Fumonisins; fungi; maize (*Zea mays* L.); mycotoxins; silo bag; storage

INTRODUCTION

Several health problems, such as immunological disorders, hematological changes, estrogenic effects, nephrotoxicity, and hepatotoxicity, may result from the ingestion of mycotoxins (*1*, *2*). Multiple variables could possibly be responsible for the contamination of grains with mycotoxins in the field, at harvest, and during transfer to storage.

The use of silo bags for storing grain for human consumption has been adopted in recent years in Argentina; therefore, it is important to study the effect of this type of storage on grain quality, and due to their low cost, their use is increasing among farmers. Postharvest storage of grains in silo bags and the prevailing factors such as moisture content and water activity (a_w) are key determinants of the impact fungi may have on the grain quality including the presence of mycotoxins (*3*). However, in Argentina, whether this practice can affect the fungal and mycotoxin contamination of maize (*Zea mays* L.) grain has not been studied systematically.

The objective of this study was to analyze fumonisin contamination in maize grain silo bags in the conditions of the three principal maize producing provinces of Argentina to determine how this kind of storage affects contamination and if differential storage durations or times of year of silo bag closing and opening are factors that could modify it.

MATERIALS AND METHODS

Maize silo bags, prepared following good agricultural practices, especially harvest moisture content, from the maize producing provinces of Buenos Aires, Córdoba, and Santa Fé, were monitored during their storage. Silo bags were 75 m long and 2.5 m in diameter, with a capacity of 180 to 220 tn, and the polyethylene used was 250 μm thick. Storage durations of 163 silo bags analyzed ranged from 120 to 226 days, closures ranging from April to June and openings from September to November, ensuring that no slits or tears occurred. Moisture content, a_w, molds and yeasts present, and fumonisins (B_1 and B_2, and total fumonisins, considered here as the sum of B_1 and B_2) were compared at the moment of closing and later opening of the silo bags. The effect of storage on fumonisin contamination, the influence of dates of closing and opening, and different storage durations was analyzed.

* Corresponding author. Tel/Fax: 54 02323 425946; E-mail: fundacion@ictbdelacruz.org.ar.

[†] Fundación de Investigaciones Científicas Teresa Benedicta de la Cruz.

[‡] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires.

[§] Universidad Nacional de Luján.

[||] Consejo Nacional de Investigaciones Científicas y Técnicas.

[⊥] Departamento de Ingeniería Química, Universidad de Buenos Aires.

[#] Instituto de Cálculo, Universidad de Buenos Aires.

[▽] Departamento de Industrias y Química Orgánica, Universidad de Buenos Aires.

10.1021/jf803609c CCC: $40.75
Published on Web 03/03/2009

Table 1. Sample Number, Moisture Content, a_w, Moulds and Yeasts, and Fumonisins at the Closing and Opening of Silo Bags

	moisture cont. (%)	a_w	molds (prop./g)	yeasts(CFU/g)	B_1(μg/kg)	B_2(μg/kg)	total (μg/kg)
			Silo Bag Closing				
N	*163*	*163*	*162*[a]	*163*	*163*	*163*	*163*
minimum value	12.0	0.55	2950	0[c]	5.0[b]	5.0[b]	10.0[b]
maximum value	15.9	0.70	945000	62500	15495	4902	20397
mean	13.2	0.63	47965	1646.6	2374.9	704.3	3079.1
median	13.2	0.63	21500	0[c]	1280	356	1632
positive samples			162	22	159	150	159
			Silo Bag Opening				
N	*163*	*163*	*162*[a]	*163*	*163*	*163*	*163*
minimum value	12.0	0.55	1650	0[c]	5.0[b]	5.0[b]	10.0[b]
maximum value	14.0	0.74	285500	227500	23668	7440	31108
mean	13.3	0.64	38519	2208.0	3287.2	951.27	4238.5
median	13.3	0.65	20400	0[c]	187.0	551	2495.1
positive samples			162	18	160	157	160

[a] In one sample, this variable was not quantified. [b] Assigned value. [c] Assigned value (yeasts not detected).

Sampling. For each silo bag, sampling was performed during the grain flow at the moment of packing and unpacking the silos, taking portions of 100 g or less to form a composite sample, and then reducing at random to a 5 kg sample, applying a quartering technique. Every 30 tons, 40 incremental samples were taken, and the aggregate sample varied from 24 to 34 kg depending on the silo bag capacity.

Moisture Content Measurement. Moisture content was determined in triplicate after the milling of the sample by drying for 1 h at 130 °C, 1.5 to 2 g of sample, according to the International Norm ISO (*4*).

Water Activity Measurement. An AquaLab CX-2 m (Decagon Devices Inc. Pullman, Washington, USA) was used to measure a_w. The operational specifications of this device were as follows: 5 to 43 °C, 20−85% relative moisture content, an infrared temperature sensor, and another that was a refrigerated mirror to determine condensation at the dew point. Range of measurement of a_w, 0.030 to 1.000; accuracy, ± 0.003 a_w; resolution, ± 0.001 a_w.

Measurements were done at 20 °C. The device was calibrated with standards of saturated saline solutions of NaBr (a_w = 0.582), NaCl (0.751), and KCl (0.851), within the range of a_w expected for maize samples. Three successive measurements were performed, and a calibration curve was done to ensure repeatibility of the measurement. Using the read values of the solutions, by means of linear regressions (R^2 greater than 0.999), we obtained the estimated equations with which the values of a_w readings of the equipment were calculated.

Isolation and Identification of Fungi. Fungi were isolated using decimal dilutions by triplicate in (*5*) yeast extract glucose chloramphenicol agar (YGC). The fungi were identified according to Nelson et al. (*6*), Pitt and Hocking (*5*), and Watanabe (*7*). Fungi were quantified as propagules per gram and yeasts as colony forming units (CFU) per gram.

Fumonisin Analysis. Corn subsamples were analyzed to determine the presence of fumonisins B_1 and B_2 according to the method of Sydenham et al. (*8*). Fifty gram subsamples were extracted with 100 mL of methanol/water (3:1) by blending for 3 min at high speed with an Osterizer blender. The extract was filtered through Whatman No. 4 paper. An aliquot (10 mL) was applied to conditioned strong anion exchange (SAX) (Merck, Darmstad, Germany) solid-phase extraction cartridges, previously conditioned with methanol/water (3:1) followed by methanol. The SAX cartridges were washed to remove corn-intrinsic compounds and the fumonisins selectively eluted with an acetic acid/methanol solution (1:99). The eluates were collected and evaporated to dryness in a 40 °C water bath under vacuum and the residue redissolved in methanol (100 μL). The HPLC system used was an Agilent 1100 series, that included a G1322A degasser, a G1313A autosampler, a G1321A fluorescence detector, G1311A quaternary pump, and a G1316A temperature controller. An aliquot was derivatized online with a solution of 40 mg *o*-phthalaldehyde in 1 mL of methanol, diluted with 5 mL of 0.1 M disodium tetraborate solution, and 50 μL of 2-mercaptoethanol. Within 1 min of reaction time, the derivatized solution was injected into the HPLC system. A 250 mm × 4 mm i.d., 5 μm, Lichrosorb C18 reverse phase column and a 4 mm × 4 mm i.d. guard column of the same phase were used. The mobile phase was methanol/0.1 M sodium dihydrogen phosphate (77:23, v/v), adjusted to pH 3.3 with orthophosphoric acid. The flow rate was 1 mL/min. Fluorescence excitation and emission wavelengths were set at 335 and 440 nm, respectively. Retention times of $B_1$and B_2 were 7 and 15.4 min, respectively.

Identification and quantification of the fumonisins were observed by comparison of the retention times and peak areas in the samples with those observed for standards (Medical Research Council, Tygerberg, South Africa). Detection and quantification limits were 10 and 18 μg/kg for fumonisin B_1 and 6 and 30 μg/kg for B_2 (calculated as signal-to-noise ratio = 3:1 and 5:1 for detection and quantification limits, respectively). Fumonisins average recoveries (3 replicates) were, at a level of 100 μg/kg, 94.5% for B_1 (CV% = 21.8) and 92.5% for B_2 (CV% = 23.5), at a level of 650 μg/kg, 94.6% for B_1 (CV% = 12.8), and at a level of 200 μg/kg, 93.1% for B_2 (CV% = 17).

Statistical Analysis. The Statistix 2000 software package (*9*) was used to obtain descriptive statistics, box plots, and to make comparisons between silo bags opening and closing. Because of the fact that the mycotoxin contamination distribution was not normal, robust estimators as the median were also used. The asymptotic normal test for paired observations was used to compare variables at the moment of closure and opening of all silo bags.

RESULTS AND DISCUSSION

Comparison of the Parameter Values between Closure and Opening Times of Silo Bags. The majority of the samples were contaminated with fumonisins B_1 and B_2 (**Table 1**), as occurs widely around the world in corn (*1*, *8*, *10*).

At the moment the silo bags were closed, the mean moisture content and a_w were 13.2% and 0.63, respectively. Molds were present in all samples, whereas yeasts were only present in 22 of the 163 samples (**Table 1**). The majority of the samples were contaminated with fumonisins B_1 and B_2 at the closing of the silo bags.

When the silo bags were opened, the mean moisture content had increased to 13.3% and the mean of a_w to 0.64. As in the case of silo bags closing, molds were present in all samples, whereas yeasts were only present in 18 of the 163 samples, and the majority of the samples were contaminated with fumonisins B_1 and B_2.

Comparing the moments of closing and opening of the silo bags, the median total fumonisin contamination at the closing was 1632 μg/kg, whereas the mean was 3079 μg/kg. The median total fumonisin contamination at opening was 2495 μg/kg, whereas the mean was 4238.5 μg/kg (**Table 1**). The difference between mean and median values observed was due to the presence of outliers (**Figure 1**).

The asymptotic normal test for paired observations was performed to compare the means at the moment of closing and

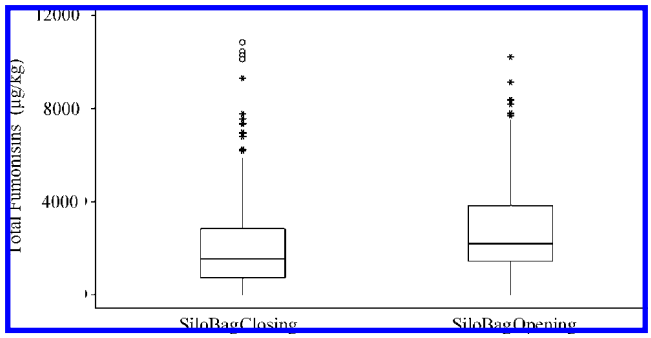

Figure 1. Box plots of total fumonisins at the closing and opening of silo bags.

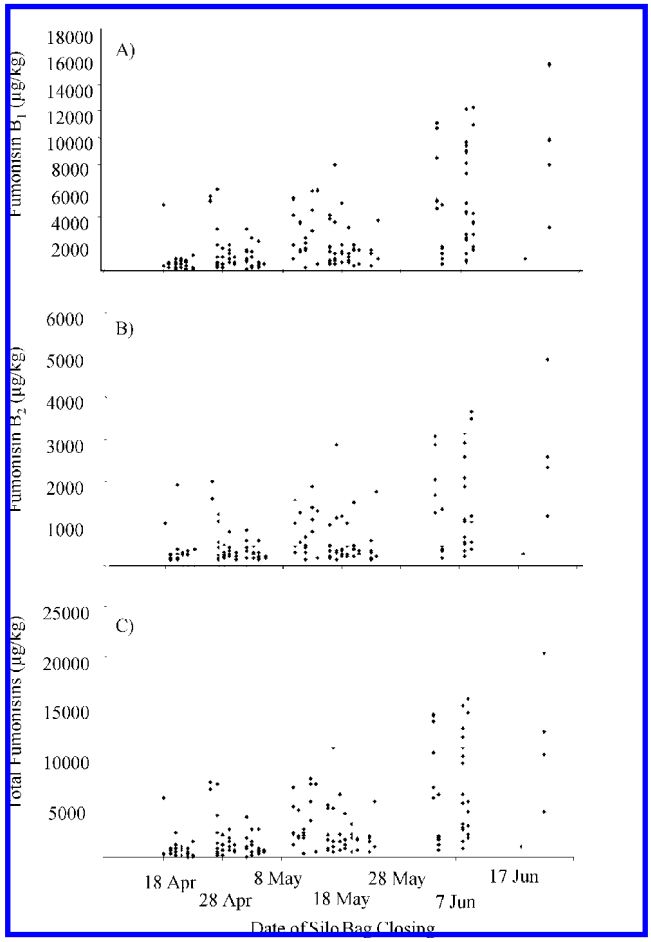

Figure 2. Dates in which silo bags were closed and the presence of fumonisins (**A**) B_1, (**B**) B_2, and (**C**) total (μg/kg).

opening. The alternative hypothesis considered were that the difference between means at the silo bag opening and closing moments was less than zero in the case of molds and greater than zero in the case of all other variables. The hypotheses were stated in this way in order to determine whether the silo bags had decreased in quality. The confidence intervals of asymptotic level 0.95 for the difference between the means were also obtained

Moisture content and a_w increased slightly from the practical point of view, as is shown in the confidence intervals that were (0.009, 0.14) and (0.006, 0.016), respectively, although statistically the differences were significant. The *p*-values were 0.0129 for moisture content and <0.0001 for a_w. Molds and yeasts did not differ significantly (*p*-values: 0.1170 and 0.3632, respectively). Fumonisins B_1, B_2, and total had increased significantly, the *p*-values being less than 0.0001.

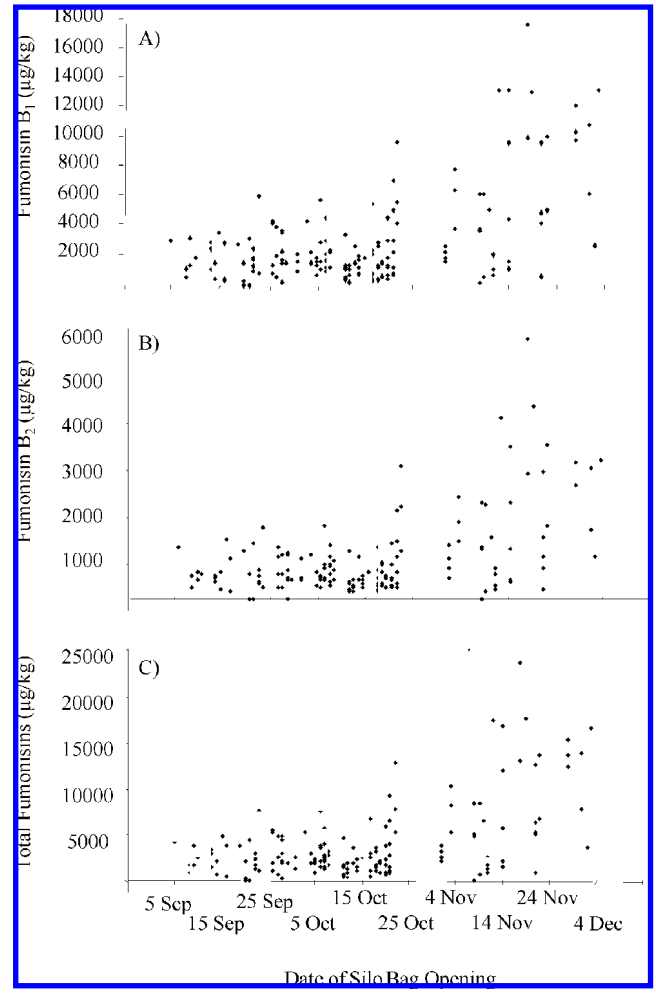

Figure 3. Dates in which silo bags were opened and the presence of fumonisins (**A**) B_1, (**B**) B_2, and (**C**) total (μg/kg).

During grain storage, a succession of different fungal species may develop according to the storage conditions and the microenvironment formed within the storage facilities (*11*). Some species are able to grow at lower a_w values and as they become established create microenvironments with higher temperature and moisture content where other molds can develop (*12*). Therefore, it is likely that one would observe, as mentioned before, a slight increase in moisture content and a_w after a period of storage. The increase detected in fumonisins at silo bag opening may be indicative of the accumulation by toxigenic molds in the storage process.

Moment of Silo Bag Closure. Analyzing the period of the year of silo bags closing, from April to June, bags closed later presented a higher level of contamination with fumonisins B_1 and B_2, compared to those closed earlier (**Figure 2A** and **B**). It was also observed that the values of total fumonisin contamination of those silo bags closed later in the year showed a greater dispersion (**Figure 2C**).

Moment of Silo Bag Opening. Considering the period of the year of silo bag opening, from September to the beginning of December, it was found that silo bags opened later in the year presented higher fumonisins B_1 and B_2 contamination levels, compared to those opened earlier (**Figure 3A** and **B**). Similarly to what occurred in the case of silo bag closings, the values of total fumonisin contamination of those silo bags opened later showed a greater dispersion (**Figure 3C**).

Storage Duration. Storage durations in the silo bags analyzed ranged from 120 to 226 days. Plotting the difference between

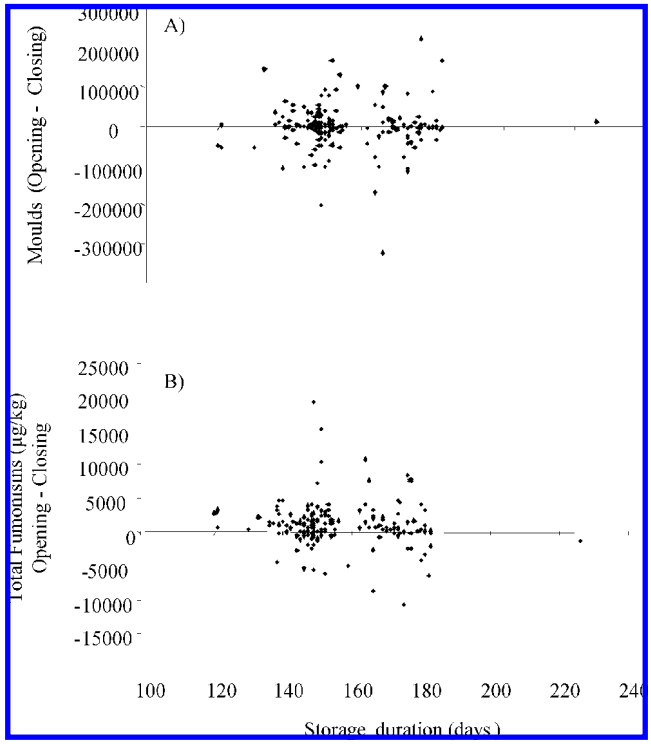

Figure 4. Difference between silo bag opening and closing of (**A**) molds and (**B**) total fumonisin contamination and days of storage duration.

silo bag opening and closing of molds and total fumonisin contamination against days of storage duration, no relationship was apparent (**Figure 4A** and **B**).

The contamination levels recorded at the closing of the silo suggest contamination with molds and fumonisins to be more dependent on the grain conditions at the moment of entrance to the silo bags than on the duration of storage. In fact, initial and final total fumonisins present a correlation coefficient of 0.69. Evidently the greatest changes occur at the beginning of storage, and after this period, a plateau seems to have been reached so that longer storage durations do not modify the studied variables.

From the data analyzed, it can be concluded that, in the conditions explored in the present study, the level of fumonisin contamination in maize grains stored following good agricultural practices in silo bags increased significantly. Moisture content and a_w increased slightly, although significantly, whereas mold propagules/g and yeasts CFU did not present significant differences between silo bag opening and closing. In the range of storage durations analyzed, longer durations did not significantly affect any of the variables studied, probably due to the fact that during the first stages of storage the greatest changes occurred so that longer storage durations did not modify these variables. A more extensive study, exploring duration from the moment of closure and continuing for shorter periods, would need to be undertaken to increase understanding of storage in silo bags in Argentina.

The date at which silo bags were closed and later opened affected the level of fumonisin contamination. This finding is of great relevance as it had not been previously reported for the environmental conditions in Argentina.

ABBREVIATIONS USED

a_w, water activity; YGC, yeast extract glucose chloranphenicol agar; CFU, colony forming units.

ACKNOWLEDGMENT

We acknowledge the technical assistance provided by Gabriela Cano and Daniela Taglieri

LITERATURE CITED

(1) FAO/WHO. *Mycotoxins*; Evaluation of certain mycotoxins; WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper 74; FAO: Geneva, Switzerland, **2001**.

(2) Pacin, A. Micotoxicosis: agente causal, acción biológica, manifestaciones clínicas y epidemiología. In *Jornada Nacional sobre Micotoxinas y Micotoxicosis*. Instituto de Investigaciones Agropecuarias Estación Experimental La Platina, Olavarría M. R.M. Muñoz, Programa Postcosecha: Santiago de Chile, Chile, 1989, p 45−76.

(3) Magan, N.; Aldred, D. Post-harvest control strategies: Minimizing mycotoxins in the food chain. *Int. J. Food Microbiol*. **2007**, *119*, 131–139.

(4) ISO 6540. Maize: Determination of Moisture Content (On Milled Grains and on Whole Grains). http://www.iso.org (accessed Feb 11, 2009).

(5) Pitt, J. I.; Hocking, A. D. *Fungi and Food Spoilage*; Blackie Academic & Professional: London, 1997.

(6) Nelson, P. E.; Toussoun, T. A.; Marasas, W. F. O. *Fusarium Species: An Illustrated Manual for Identification*; The Pennsylvania State University Press: University Park, PA, 1983.

(7) Watanabe, T. *Pictorial Atlas of Soil and Seed Fungi. Morphologies of Cultured Fungi and Key to Species*; CRC Press: Boca Raton, FL, 2002.

(8) Sydenham, E. W.; Shepard, G. S.; Thiel, P. G.; Marasas, W. F. O.; Rheeder, J. P.; Peralta Sanhueza, C. E.; González, H. H. L.; Resnik, S. L. Fumonisins in Argentinian field-trial corn. *J. Agric. Food Chem*. **1993**, *41* (6), 891–895.

(9) *Statistix 7*; Analytical Software: Tallahassee, FL, 2000.

(10) Shephard, G. S.; Thiel, P. G.; Stockenström, S.; Sydenham, E. W. Worldwide survey of fumonisin contamination of corn and corn-based products. *J. AOAC Int*. **1996**, *79*, 671–687.

(11) Wicklow, D. T. The Mycology of Stored Grain: An Ecological Perspective. In *Stored Grain Ecosystems*; Jayas, D. S., White, N. D. G., Muir, W. E., Eds.; Marcel Dekker: New York, 1995; p 197−249.

(12) Hocking, A. D. Microbial Facts and Fictions in Grain Storage. In *Stored Grain in Australia 2003*. Proceedings of the Australian Postharvest Technical Conference; Wright, E. J., Webb, M. C., Highley, E., Eds.; CSIRO: Canberra, Australia, 2003; p 55−58.

Received for review November 19, 2008. Revised manuscript received February 12, 2009. Accepted February 12, 2009. We acknowledge the financial support of the Consejo Nacional de Investigaciones Científicas y Técnicas, Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Universidad de Buenos Aires, Fundación de Investigaciones Científicas Teresa Benedicta de la Cruz, and the MYCOTOX project, The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries, ref ICA4-CT-2002-10043.

JF803609C