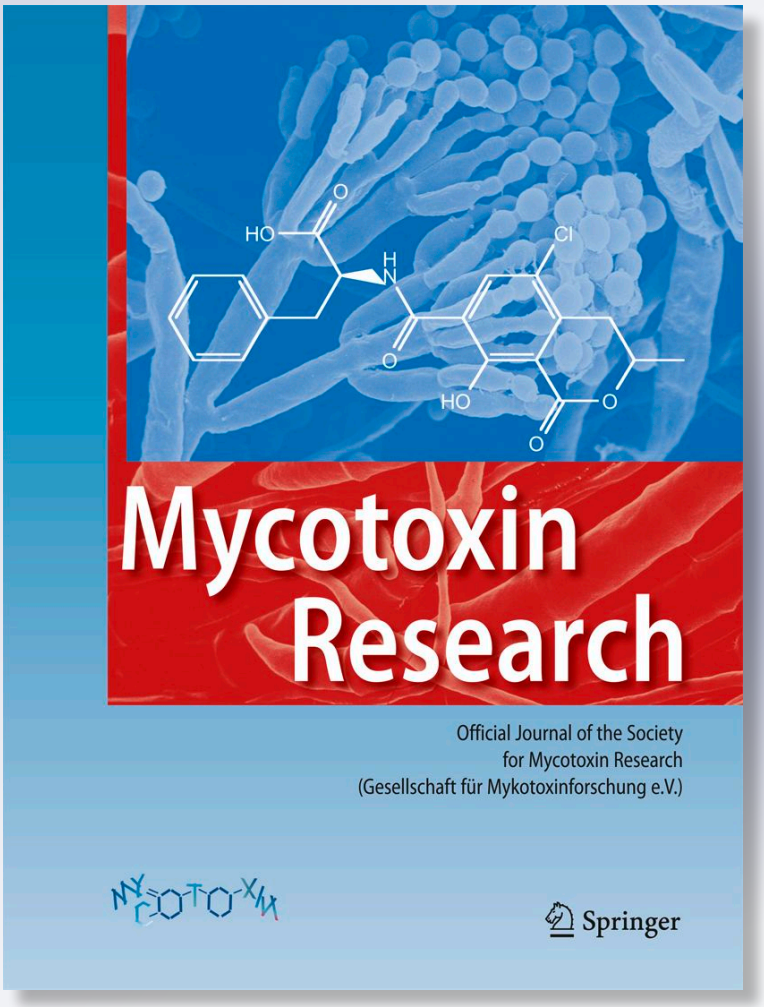
Natural occurrence of alternariol and alternariol monomethyl ether in soya beans

M. S. Oviedo, G. G. Barros, S. N. Chulze & M. L. Ramirez

Mycotoxin Research

ISSN 0178-7888
Volume 28
Number 3

Mycotoxin Res (2012) 28:169-174
DOI 10.1007/s12550-012-0132-0



Springer

Mycotoxin Res (2012) 28:169–174
DOI 10.1007/s12550-012-0132-0

ORIGINAL PAPER

Natural occurrence of alternariol and alternariol monomethyl ether in soya beans

M. S. Oviedo · G. G. Barros · S. N. Chulze · M. L. Ramirez

Received: 16 February 2012 / Revised: 29 May 2012 / Accepted: 30 May 2012 / Published online: 14 June 2012

Abstract The natural occurrence of alternariol (AOH) and alternariol monomethyl ether (AME) in soya beans harvested in Argentina was evaluated. Both toxins were simultaneously detected by using HPLC analysis coupled with a solid phase extraction column clean-up. Characteristics of this in-house method such as accuracy, precision and detection and quantification limits were defined by means of recovery test with spiked soya bean samples. Out of 50 soya bean samples, 60% showed contamination with the mycotoxins analyzed; among them, 16% were only contaminated with AOH and 14% just with AME. Fifteen of the positive samples showed co-occurrence of both mycotoxins analyzed. AOH was detected in concentrations ranging from 25 to 211 ng/g, whereas AME was found in concentrations ranging from 62 to 1,153 ng/g. Although a limited number of samples were evaluated, this is the first report on the natural occurrence of *Alternaria* toxins in soya beans and is relevant from the point of view of animal public health.

Keywords *Alternaria alternata* · Alternariol · Alternariol monomethyl ether · Soya bean

Introduction

Over time, rural landholders in Argentina have farmed the land more and more intensively. One of the most land-intensive crops is soya beans, which serve as an important source of proteins and vegetable oil and is also one of the main ingredients in food products such as soy sauce, tofu, and soymilk. Soya beans are also used to manufacture animal feed and they serve as a raw material for generating biofuels. Argentina is one of the world's largest exporters of soya beans and also ranks third in the world in soya bean production. Argentina produces approximately 50,000,00 tons of soya beans per year, and the Cordoba Province provides about 26% of the national production (MAGyP 2011).

Saprophytic and parasitic fungi may be borne in or on the soya beans, pods, and flowers. The occurrence of fungi in seeds has received far more attention than the occurrence of fungi in pods and flowers. Infected seeds and infected seedlings developing from them represent greater economic risk in soybean production, and contamination with mycotoxins represents a health risk to humans and animals. *Alternaria* and *Fusarium* species are the most commonly isolated fungi from soya beans in Argentina and in others regions of the world (Barros et al. 2011; Boca et al. 2003; Broggi et al. 2007; Gally et al. 2006; Roy et al. 2000; Villarroel et al. 2004). The most common *Alternaria* species found on soya bean is *A. alternata* (Barros et al. 2011; Broggi et al. 2007).

Alternaria species are well known for the production of toxic secondary metabolites, some of which are powerful mycotoxins that have been implicated in the development of human esophageal cancer (Thomma 2003). Among these metabolites with mammalian toxicity are alternariol (AOH) and alternariol monomethyl ether (AME) (Logrieco et al. 2009; Ostry 2008). AOH and AME have cytotoxic, genotoxic, estrogenic and mutagenic effects in vitro (Brugger et al. 2006; Fehr et al. 2009; Lehmann et al. 2006; Wollenhaupt et al. 2008), and there is some evidence of carcinogenic effects (Yekeler et al. 2001). These mycotoxins are produced

M. S. Oviedo · G. G. Barros · S. N. Chulze · M. L. Ramirez (✉)
Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas Fco-Qcas y Naturales, Universidad Nacional de Rio Cuarto,
Ruta 36 Km 601,
5800 Río Cuarto, Córdoba, Argentina
e-mail: mramirez@exa.unrc.edu.ar

by *Alternaria* species on wheat, tomatoes, sorghum, pecans, sunflower and cotton (Ostry 2008; Scott 2001).

Alternaria mycotoxins are usually extracted from food with organic solvents. Clean-up procedures include solvent partitioning and solid phase extraction (SPE). Detection can be done after separation by thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) methods with fluorescence detection (FLD), UV-detection and diode array detection (DAD). Most of the recent analytical methods used for the analysis of *Alternaria* toxins include reversed phase HPLC or ultra performance liquid chromatography (UPLC) coupled to single MS or tandem MS (MS/MS) (EFSA 2011).

Alternaria toxins have recently received much attention, both in research programs and in risk assessment studies. At present, no statutory or guideline limits set for *Alternaria* mycotoxins have been set by regulatory authorities (FAO 2004). Current data on the natural occurrence of *Alternaria* toxins point to low human dietary exposure. Further studies are necessary to develop strategies for safe food and feed supplies by developing detection methods, identifying the risk of *Alternaria* mycotoxins in the production chain, determining the critical points, and developing preventive measures. During the last year the European Commission asked the European Food Safety Authority to review the safety of *Alternaria* toxins in food and feed. The panel on contaminants in the food chain used the threshold of toxicological concern (TTC) approach value to assess the relative level of concern for dietary exposure of humans to these mycotoxins. For the genotoxic *Alternaria* toxins, AOH and AME, the estimated chronic dietary exposure exceeded the relevant TTC indicating a need for additional toxicity data (EFSA 2011).

Apart from the substrate, mould growth and mycotoxin production depends on temperature and water activity (a_W). Previously, studies in relation to ecophysiological parameters (a_W and temperature) on in vitro (soya bean based media) and in situ (irradiated soya bean) growth and AOH and AME by two strains of *A. alternata* isolated from soya beans in Argentina have already been carried out (Oviedo et al. 2009, 2011). The a_W and temperature range used in both studies simulate those occurring during grain ripening. Maximum growth rate and mycotoxin production were obtained at 0.98 a_W at 25 and 30°C. Also, we were able to demonstrate the growth and AOH and AME production under marginal or sub-optimal temperature and a_W conditions. This information can be important since improper storage conditions accompanied by elevated temperature and moisture content in the grain can favor further mycotoxin production and lead to reduction in grain quality and also could present a hazard if the grain is used for human consumption or animal feedstuff.

The aim of this work was to evaluate the natural occurrence of AOH and AME on soya beans harvested in Argentina.

Materials and methods

Chemicals and materials

Acetonitrile, methanol (both HPLC grade) and glacial acetic acid for organic residue analysis were purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). Ultrapure water was produced by a Millipore Milli-Q system (Millipore, Bedford, MA, USA). AOH and AME toxin were purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA). C_{18} column were obtained from Waters (Waters, Milford, MA, USA), and grade 4 paper filters from Whatman (Maidstone, UK).

Standard preparation and calibration curve

AOH and AME were purchased from Sigma, in crystallized form. A mixed stock solution of AOH and AME containing 0.5 mg/ml of each toxin was prepared gravimetrically as follows: 1.0 mg of each toxin was weight in an analytical balance (capable of weighing 0.1 mg), then transferred to a glass stoppered volumetric flask (2 ml); methanol was added to about two-thirds full and the toxin crystals dissolved, before filling with methanol up to the mark. Six spiking solutions were prepared at concentrations of 0.005, 0.01, 0.05, 0.1, 0.2 and 0.3 mg/ml for each toxin, diluting aliquots of the mixed stock solution with the appropriately volumes of methanol. Then a mixed standard solution was prepared in methanol containing 5.0 μl/ml AOH and AME by appropriately diluting an aliquot of the mixed stock solution with methanol. Five mixed working calibrant solutions (0.5, 1.0, 2.0, 3 and 10 μl/ml) were prepared by diluting an aliquot of the standard solution with the appropriate volume of LC mobile phase (acetonitrile:water, 25/75, v/v). The calibration solutions showed correlation coefficients with a linear relationship better than 0.995.

Samples

Fifty soya bean samples (harvest 2006/2007 and 2007/2008) were obtained from a local grain storage company upon arrival. Each whole sample was around 25–30 tons, which represented the truckload capacity. The soya beans were taken in six different truck positions using a vacuum sampling device in order to obtain an aggregate sample of 10 kg of randomized seeds. This sample was homogenized and sub-samples of 1 kg were taken, finely milled by using a Romer mill (Romer, Union, MO, USA), mixed well and

stored at -20 °C until analysis. The soya beans were grown in fields located in two provinces of Argentina (Córdoba, n=49, and San Luis, n=1).

Toxin extraction and clean-up

A sample of 20 g was extracted with 2 g NaCl, 30 ml hexane and 50 ml acetonitrile:methanol:water at 45/10/45 (v/v/v, adjusted to pH 3 using 1.0 M HCl) by shaking in a orbital shaker for 60 min. The extract was filtered through Whatman no. 4 filter paper, and 10 ml filtrate was collected. A 2.5-ml sample of the aqueous phase extract was applied to a C_{18} column (10 ml capacity, containing 500 mg C_{18} sorbent; Waters, Milford, MA, USA) fitted to a Supelco solid-phase extraction (SPE) manifold (Supelco, Bellefonte, PA), previously conditioned by the passage of 6 ml acetonitrile until air comes through the column. Toxins were eluted with 2 ml acetonitrile/glacial acetic acid at 99/1 (v/v). The eluted extract was collected in a 4-ml screw-cap amber vial and evaporated under a moderate stream of nitrogen at 50°C in a heating block (Thermo Scientific, Rockford, IL, USA). The residue after clean-up was dissolved in 200 μl methanol by vortexing for 1 min and diluted with 200 μl acetonitrile/NaH_2PO_4 (0.027 M) (25/75, v/v) and stored at 4°C until HPLC analysis.

HPLC analysis

The HPLC system consisted of an HP1100 pump (Hewlett Packard, Palo Alto, CA) connected to an HP 1100 series

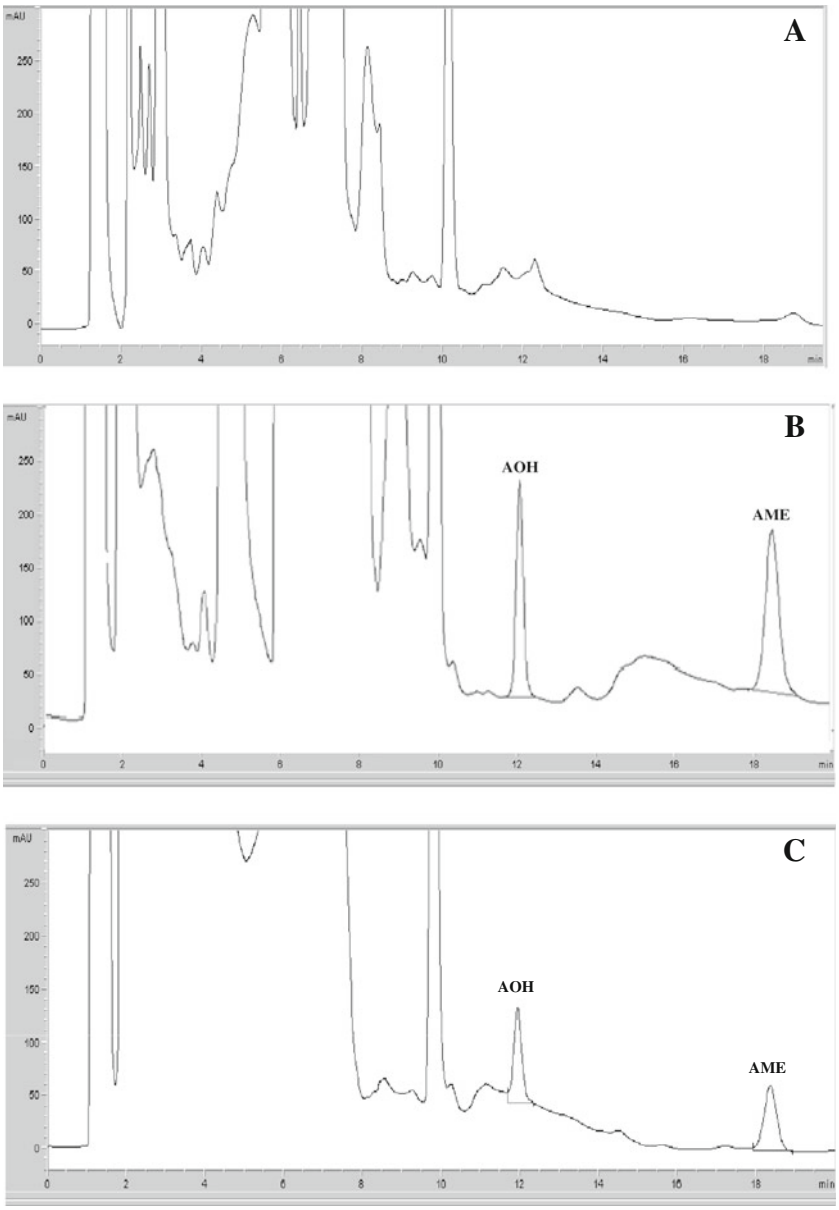


Fig. 1 HPLC chromatograms (**a**) blank soya bean sample, (**b**) fortified soya bean with 100 ng/g AOH and AME respectively, (**c**) naturally contaminated soya beans with 42 ng/g and 50 ng/g of AOH and AME respectively

Table 1 Results of the recovery experiment for alternariol and alternariol monomethyl ether

Spiking level (ng/g)	AOH		AME	
	Recovery ± SD (%)	RSD (%)	Recovery ± SD (%)	RSD (%)
3,000	75.65±3.62	4.79	104.53±12.61	12.06
2,000	100.83±19.18	19.00	98.53±8.26	8.38
1,000	101.99±28.97	28.40	98.04±6.71	6.84
500	96.74±26.51	27.40	97.01±1.91	2.74
100	98.66±27.71	21.30	98.34±1.00	2.05
50	95.50±20.45	20.90	97.40±1.88	3.35
	94.90±21.07	20.30	98.98±5.40	5.90

AOH alternariol, *AME* alternariol monometyl ether, *SD* standard deviation (n=3), *RSD* relative standard deviation

variable wavelength detector and a data module Hewlett Packard Kayak XA (HP ChemStation rev. A.06.01). Chromatographic separations were performed on a SymmetryShield™ C_{18} column (100 mm × 4.6 mm, 5-μm particle size, Waters) connected to a SecurityGuard™ guard column (20 mm × 4.6 mm, Phenomenex) filled with the same stationary phase. The mobile phase consisted of two consecutive isocratic mobile phase mixtures containing acetonitrile/water (25/75, v/v, solvent A) acetonitrile/water (50/50, v/v, solvent B). Solvent A was pumped for 3.5 min at 1.0 ml min^{-1}, and then solvent B was pumped for 16.5 min at 1.0 ml min^{-1}. The detector was set at 256 nm for AOH and AME. Injection volume was 50 μl and the retention time of AOH and AME were 11.9 and 18.3 min, respectively. Quantification was relative to external standards of 0.5, 1.0, 2.0, 3 and 10 μg/ml in acetonitrile/water (25/75, v/v).

Confirmation of the toxins in some positive samples was achieved by using a Waters Alliance HPLC system with e2695 separation module and Waters 2998 PDA detector and the signal were processed by Empower™ software. We used the same column and HPLC condition that were described above. The PDA detector was set at 256 nm, because this wavelength corresponds to the maximum absorbance for AOH and AME. Reference spectra were acquired during the elution of associated standards and used for peak identification, then comparison between spectra and retention time of standards and natural contaminated samples was made.

Recovery experiment

Recovery experiments were performed in triplicate by spiking 20 g ground blank soya bean samples with AOH and AME toxins at levels of 50, 100, 500, 1,000, 2,000 and 3,000 ng/g. Spiked samples were left overnight at room temperature to allow solvent evaporation prior to proceed with the extraction step.

Statistical analysis

To determine differences among *Alternaria* toxin contents in relation to the harvest seasons, the nonparametric test Kruscal Wallis one-way analysis of variance was used. The study was made using SigmaStat for Windows version 2.03 (SPSS, Chicago, IL, USA). Statistical significance was determined at the level of $P \leq 0.05$.

Results and discussion

Numerous methods have been developed for analysis of AOH and AME in different agricultural commodities (Logrieco et al. 2009; Ostry 2008; Scott 2001), but not for determining these mycotoxins in soya bean. Solid-phase extraction columns have been used for extraction and clean-up of AOH and AME in apple juice and wheat (Scott 2001; Scott and Kanhere 2001). The methodology applied in this study was based on the method described by Solfrizzo et al. (2004), with some modifications. The modifications included the addition of NaCl during the extraction stage, this procedure reduced interfering compounds and also decreased both noise and peak numbers due to the impurities present in the matrix that were observed in the chromatograms of blank and spiked soybean samples. Also, hexane was added to the extraction mixture, as a defatting solvent. Similar chromatogram profiles were

Table 2 Natural occurrence of *Alternaria* mycotoxins in soya beans

Number of samples (n=50)	Toxins (ng/g)	
	AOH	AME
15	25–141 (72)[a]	75–1,153 (363)[a]
8	25–211 (70)[a]	ND
7	ND	62–483 (277)[a]

AOH alternariol, *AME* alternariol monometyl ether, *ND* not detected

[a] Values in parentheses are means

obtained for the blank, spiked samples, and test samples (Fig. 1).

Recovery experiments were carried out in triplicate with six different spiking levels of both mycotoxins: results are presented in Table 1. Mean recoveries for AOH and AME from soya beans spiked at levels between 50 and 3,000 ng/g were 94.90% and 98.98%, respectively, with a within-laboratory relative standard deviation (RSD) of 20% and 5.9% for AOH and AME respectively. Detection limits (LOD) were 8 and 16 ng/g for AOH and AME, respectively, based on the signal-to-noise ratio (3:1) and the limit of quantitation (LOQ) was established as three-times the limit of detection (24 and 48 ng/g).

The use of SPE clean-up columns for extract purification was convenient for time saving and reduction of clean-up steps. Although no collaborative validation of the method presented herein has been performed, the procedure adopted in this study has been shown to be accurate and precise for determining AOH and AME in the substrate evaluated.

Data on natural occurrence of AOH and AME are shown in Table 2. Out of 50 soya bean samples, 60% showed contamination with the mycotoxins analyzed; among them, 16% were only contaminated with AOH and 14% just with AME. Fifteen of the positive samples showed co-occurrence of both mycotoxins analyzed. AOH was detected in concentrations ranging from 25 to 211 ng/g, whereas AME was found in concentrations ranging from 62 to 1,153 ng/g. Confirmation of AOH and AME in naturally contaminated soya bean samples was performed by comparing the retention time and UV spectrum with authentic standard. Samples naturally contaminated with AOH and AME showed a similarity index of 0.99 when the UV spectrum of the toxins were compared with the UV spectrum of the authentic standards of AOH and AME. The fortified soya bean sample extracts showed symmetric AOH and AME peaks at a retention time of 11.9 and 18.3 min respectively. Comparison between the two harvest seasons did not show significant differences ($P \leq 0.05$) in *Alternaria* toxins content.

This is the first report on the natural occurrence of *Alternaria* mycotoxins in soya beans and also confirms that AOH and AME are produced during soya bean development in the field. These data agree with previous studies in which we have demonstrated that the environmental conditions (a_W and temperature) optimum for growth and mycotoxin production by *A. alternata* on soya bean-base media on irradiated soya beans were similar to those occurring during soya bean development in the field until harvest (Oviedo et al. 2009, 2011).

Data on the presence of mycotoxins in soya beans and their by-products in Argentina are limited to *Fusarium* mycotoxins (deoxynivalenol, T-2 toxin, HT-2 toxin, zearalenone and fumonisins) and *Aspergillus* mycotoxins (aflatoxins and ochratoxins). Deoxynivalenol, zearalenone, ochratoxins, fumonisins, aflatoxins and T-2 toxin have been found as natural contaminants of soya beans and their by-products in Argentina (Barros et al. 2008, 2011, 2012; Boca et al. 2003; Lopez et al. 2006a, b). *Alternaria* mycotoxins should not be underestimated, since they can co-occur with other mycotoxins whose toxicity has been widely demonstrated and synergism among the toxins can occur.

During the last 5 years numerous studies dealing with AOH and AME toxicity have been published. Both mycotoxins have been reported to have genotoxic, mutagenic and carcinogenic effects (Brugger et al. 2006; Fehr et al. 2009; Lehmann et al. 2006; Wollenhaupt et al. 2008; Yekeler et al. 2001). Recently, Tiemann et al. (2009) have demonstrated that AOH and AME, at similar concentration levels found in the present study, negatively affected progesterone synthesis in porcine granulosa cells in vitro. In view of the fact that granulosa cells directly influence the metabolic and structural growth of the oocyte (Albertini et al. 2001), exposure to AOH or AME may eventually affect reproductive performance by interfering with follicular development in swine and possibly other mammalian species. Feedstuff should therefore be carefully controlled for *Alternaria* toxin content.

Beside the limited number of samples evaluated, the high frequency of natural occurrence in soya beans of *Alternaria* metabolites reported in the present study indicate that further studies on natural occurrence, effect of food processing on toxin concentration, and prevention strategies are needed. Considering the extensive use of soya beans in the manufacture of animal and human foodstuffs, the risk to both populations arising from continuing low-level exposure to AOH and AME should be taken into account. The method outlined in this study was useful to evaluated natural occurrence of these toxins in soya beans.

Acknowledgments This work was supported by the Secretaría de Ciencia y Técnica, Universidad Nacional de Río Cuarto (SECyT-UNRC). M.S.O. is a fellow of CONICET and M.L.R., G.G.B. and S.C., are members of the Research Career of CONICET.

Conflicts of interest None.

References

Albertini DF, Combelles CM, Benecchi E, Carabatsos MJ (2001) Cellular basis for paracrine regulation of ovarian follicle development. Reproduction 121:647–653

Barros G, García D, Oviedo S, Ramirez ML, Torres A, Chulze S (2008) Trichothecene analysis in soybean seeds and flour. World Mycotoxin J 1:263–266

Barros G, García D, Oviedo M, Ramirez ML, Torres A, Lattanzio V, Pascale M, Chulze N (2011) Survey of T-2 and HT-2 toxins in soybean and soy meal from Argentina using immunoaffinity clean-up and high performance liquid chromatography. World Mycotoxin J 4:189–197

Barros G, Alaniz Zanon MS, Abod A, Oviedo MS, Ramirez ML, Torres A, Chulze S (2012) Natural deoxynivalenol occurrence and genotype and chemotype determination of a field population of the *Fusarium graminearum* complex associated with soybean in Argentina. Food Addit Contam 29:293–303

Boca RT, Pacin AM, Gonzalez HHL, Resnik SL, Souza JC (2003) Soja y micotoxinas: Flora fúngica—Variedades—Prácticas agronómicas. Aceites y Grasas 4:510–515

Broggi L, González HHL, Resnik S, Pacin A (2007) *Alternaria alternata* prevalence in cereal grains and soybean seeds from Entre Rios, Argentina. Rev Iberoam Micol 24:47–51

Brugger EM, Wagner J, Schumacher DM, Koch K, Podlech J, Metzler M, Lehmann L (2006) Mutagenicity of the mycotoxin alternariol in cultured mammalian cells. Toxicol Lett 164:221–230

EFSA on Contaminants in the Food Chain (CONTAM) (2011) Scientific opinion on the risk for animal and public health related to the presence of *Alternaria* toxins in feed and food. EFSA J 9(10):2407. [97 pp.] doi:10.2903/j.efsa.2011.2407. (www.efsa.europa.eu/efsajournal)

FAO (Food and Agriculture Organization of the United Nations) Animal Production and Health, Protein Sources for the Animal Feed Industry (2004) Available at: ftp://ftp.fao.org/docrep/fao/007/…/y5019e00.pdf

Fehr M, Pahlke G, Fritz J, Christensen MO, Boege F, Altemöller M, Podlech J, Marko D (2009) Alternariol acts as a topoisomerase poison, preferentially affecting the II isoform. Mol Nut Food Res 53:441–451

Gally T, Gonzalez BA, Pastuso F (2006) Efecto conjunto de *Fusarium* sp. y *Phomopsis* sp., patógenos transmitidos por las semillas en plántulas de soja [*Glycine max* (L.) Merrill]. Rev Mex Fitopatol 24:156–158

Lehmann L, Wagner J, Metzler M (2006) Estrogenic and clastogenic potential of the mycotoxin alternariol in cultured mammalian cells. Food Chem Toxicol 44:398–408

Logrieco A, Moretti A, Solfrizzo M (2009) *Alternaria* toxins and plant disease: an overview of origin, occurrence and risk. World Mycotoxin J 2:129–140

Lopez C, Bulacio L, Amigot S, Biasoli M, Luque A, Ramadán S, Ramos L, Sortino M, Tosello M, Fulgueira C (2006) Simultaneous determination of aflatoxins, deoxynivalenol, T-2 toxin and zearalenone in Argentinian soybean. In: Advances in research on toxigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context, 15/17 March, Villa Carlos Paz, Córdoba, Argentina, pp 128

Lopez C, Bulacio L, Ramos L, Amigot S, Ramadán S, D´espósito R, Knass P, Fulgueira C (2006) Presence of T-2 toxin in raw material for food and feed. In: Advances in research on toxigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context, 15/17 March, Villa Carlos Paz, Córdoba, Argentina, pp 127

Ministerio de Agricultura, Ganaderia y Pesca, Presidencia de la Nación (2011) Sistema integrado de información agropecuaria. (http://www.siia.gov.ar/index.php/series-por-tema/agricultura) Accessed 3 December 2011

Ostry V (2008) *Alternaria* mycotoxins: an overview of chemical characterization, producers, toxicity and occurrence in foodstuffs. World Mycotoxin J 1:175–188

Oviedo MS, Ramirez ML, Barros GG, Chulze SN (2009) Impact of water activity and temperature on growth, alternariol and alternariol monomethyl ether production by *Alternaria alternata* isolated from soybean. J Food Prot 73:336–343

Oviedo MS, Ramirez ML, Barros GG, Chulze SN (2011) Influence of water activity and temperature on growth and mycotoxin production by *Alternaria alternata* on irradiated soya beans. Int J Food Microbiol 149:127–132

Roy KW, Baird RE, Abney TS (2000) A review of soybean (*Glycine max*) seed, pod and flower mycofloras in North America, with methods and a key for identification of selected fungi. Mycopathologia 150:15–27

Scott PM (2001) Analysis of agricultural commodities and foods for *Alternaria* mycotoxins. J AOAC Int 84:1809–1817

Scott PM, Kanhere S (2001) Chromatographic method for *Alternaria* toxins in apple juice. In: Trucksess MW, Pohland AE (eds) Mycotoxin protocols. Humana Press, Totowa, pp 225–234

Solfrizzo M, De Girolamo A, Vitti C, Visconti A, Van den Bulk R (2004) Liquid chromatographic determination of *Alternaria* toxins in carrot. J AOAC Int 87:101–106

Thomma BPHJ (2003) *Alternaria* spp.: from general saprophyte to specific parasite. Mol Plant Pathol 4:225–236

Tiemann U, Tomek W, Schneider F, Müller M, Pöhland R, Vanselow J (2009) The mycotoxins alternariol and alternariol methyl ether negatively affect progesterone synthesis in porcine granulosa cells in vitro. Toxicol Lett 186:139–145

Villarroel DA, Baird RE, Trevathan LE, Watson CE, Scruggs ML (2004) Pod and seed micoflora on transgenic and conventional soybean [*Glycine max* (L.) Merrill] cultivars in Mississipi. Mycopathologia 157:207–215

Wollenhaupt K, Schneider F, Tiemann U (2008) Influence of alternariol (AOH) on regulator proteins of cap dependent translation in porcine endometrial cells. Toxicol Lett 182:57–62

Yekeler H, Bitmis K, Ozcelik N, Doymaz MZ, Calta M (2001) Analysis of toxic effects of *Alternaria* toxins on esophagus of mice by light and electron microscopy. Toxicol Pathol 29:492–497