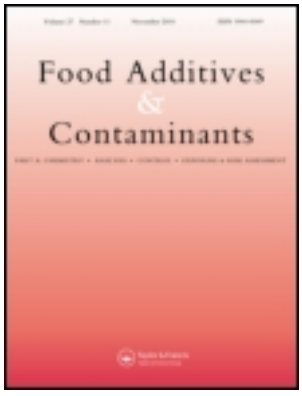

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Trichothecenes and zearalenone production by *Fusarium equiseti* and *Fusarium semitectum* species isolated from Argentinean soybean

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Fusarium equiseti and *Fusarium semitectum* represent the most abundant species in the *Fusarium* complex isolated from flowers, soybean pods and seeds in Argentina. The aim of the present study was to assess the production of major type A and type B trichothecenes (diacetoxyscirpenol, neosolaniol, T-2 toxin and HT-2 toxin, nivalenol, deoxynivalenol) and zearalenone by 40 *F. equiseti* and 22 *F. semitectum* isolates on rice culture. Mycotoxins were determined by HPLC with fluorescence detection after derivatisation with 1-anthronylnitrile for type A trichothecenes (i.e. diacetoxyscirpenol, neosolaniol, T-2 toxin and HT-2 toxin), by HPLC with UV detection for type B trichothecenes (i.e. nivalenol and deoxynivalenol), and by TLC for zearalenone. A total of 22 of 40 *F. equiseti* isolates produced diacetoxyscirpenol, nivalenol and ZEA alone or in combination, whereas only two of 20 *F. semitectum* isolates were nivalenol and ZEA producers. Both *Fusarium* species did not produce any deoxynivalenol, neosolaniol, T-2 toxin and HT-2 toxin. The variable retention in toxigenicity displayed by both fungal species suggests that these species have a saprophytic lifestyle in the soybean agroecosystem in Argentina.

Keywords: *Fusarium equiseti*; *Fusarium semitectum*; mycotoxins; trichothecenes; zearalenone; soybean

Introduction

Soybean (*Glycine max* L.), which is the main source of protein throughout the world, is largely used both as a food and as feedstuffs. In fact Argentina ranks third among soybean world producers and around 70% of the soybean harvested is processed, providing 81% of the world's exported soybean oil and 36% of soybean meal (Ministerio de Agricultura Ganadería Pesca y Alimentación de la Nación (MAGPyA) 2011).

Fusarium rot of soybeans is caused by a complex of species (Pitt and Hocking 1997), and several are known to produce a broad spectrum of toxins including type A and B trichothecenes and zearalenone (Desjardins 2006). In a previous study, the dynamic of the *Fusarium* species populations associated with soybean during crop reproductive stages was evaluated. Results showed that *F. equiseti* and *F. semitectum* were the most frequently species isolated from flowers, pods and soybean seeds, and together represented a 67% of the total *Fusarium* species isolated (Barros et al. 2011).

F. semitectum is commonly isolated from soil and from diverse aerial plant parts in tropical and subtropical areas. Although there are many reports of *F. semitectum* being implicated in various diseases, it is often not regarded as an important plant pathogen (Leslie and Summerell 2006). This fungus is known to produce mycotoxins such as trichothecenes and zearalenone (Marasas et al. 1984).

F. equiseti is a ubiquitous soil saprophyte associated with fruit rots and dead and plant tissues that also may be a pathogen on a wide range of agricultural plants (Bosch and Mirocha 1992). Secondary metabolites produced by *F. equiseti* vary in amount and toxicity. From rice cultures it was observed that this species produced type A trichothecenes, e.g. neosolaniol (NEO), diacetoxy-scirpenol (DAS), T-2 and HT-2 toxins, type B trichothecenes, e.g. nivalenol (NIV), and important non-trichothecene compounds such as zearalenone (ZEA), equisetin and fusarochromanone (Thrane 1989, 2001; Wu et al. 1990; Langseth et al. 1999; Kosiak et al. 2005).

Most analytical methods for type A trichothecenes are based on ELISA (Park and Chu 1993), gas chromatography (Croteau et al. 1994; Furlong and Valente-Soares 1995), gas chromatography coupled with mass spectrometry (MS) (Krska et al. 2001; Berthiller et al. 2005; Lattanzio et al. 2007), and HPLC. This last method is complicated by the absence of a chromophore in the molecules; however, the use of different derivatising reagents allowed the detection

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of several type A trichothecenes by fluorescence (Cohen and Botin-Muma 1992; Jiménez et al. 2000; Mateo et al. 2002; Pascale et al. 2003; Visconti et al. 2005; Lippolis et al. 2008).

In the light of the prevalence of *F. equiseti* and *F. semitectum* through the different reproductive stages in soybean development, it is relevant to characterise the toxigenic ability in order to evaluate the toxicological risk of these species in the soybean crop. Therefore, the aim of the present study was to determine the trichothecenes and zearalenone production by *F. semitectum* and *F. equiseti* populations isolated from soybean.

Materials and methods

Fungal isolates

The study included 62 isolates, 40 *F. equiseti* and 22 *F. semitectum*, characterised morphologically using the methodology proposed by Leslie and Summerell (2006). These isolates were collected in a previous study during 2007/2008 harvest season in an experimental field at the National University of Río Cuarto, Córdoba, Argentina (Barros et al. 2011) (Table 1). All isolates were maintained on Synthetisher Nährstoffarmer Agar (SNA) slants (Nirenberg 1976).

Table 1. Production of type A and B trichothecenes and zearalenone by *F. equiseti* isolates on rice culture.

Isolate	Source/stage	DAS ($\mu g\,g^{-1}$)	NEO ($\mu g\,g^{-1}$)	T-2 ($\mu g\,g^{-1}$)	HT-2 ($\mu g\,g^{-1}$)	NIV ($\mu g\,g^{-1}$)	ZEA
F5025	Flower R2	n.d.	n.d.	n.d.	n.d.	n.d.	+
F5118	Flower R2	0.4	n.d.	n.d.	n.d.	n.d.	−
F5119	Flower R2	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5120	Flower R2	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5126	Flower R2	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5130	Flower R2	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5144	Flower R2	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5027	Debris	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5114	Debris	1.5	n.d.	n.d.	n.d.	5.0	+
F5007	Pod R6	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5008	Pod R6	n.d.	n.d.	n.d.	n.d.	17.0	−
F5012	Pod R6	n.d.	n.d.	n.d.	n.d.	16.0	−
F5013	Pod R6	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5014	Pod R6	n.d.	n.d.	n.d.	n.d.	2.5	+
F5019	Pod R6	n.d.	n.d.	n.d.	n.d.	6.3	+
F5020	Pod R6	2.5	n.d.	n.d.	n.d.	n.d.	−
F5143	Pod R6	n.d.	n.d.	n.d.	n.d.	n.d.	+
F5149	Pod R6	n.d.	n.d.	n.d.	n.d.	n.d.	+
F5151	Pod R6	0.6	n.d.	n.d.	n.d.	n.d.	+
F5153	Pod R6	n.d.	n.d.	n.d.	n.d.	93.0	+
F5154	Pod R6	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5157	Pod R6	n.d.	n.d.	n.d.	n.d.	n.d.	+
F5010	Seed R6	n.d.	n.d.	n.d.	n.d.	32.1	+
F5029	Seed R6	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5042	Seed R6	n.d.	n.d.	n.d.	n.d.	10.0	−
F5065	Seed R6	n.d.	n.d.	n.d.	n.d.	17.8	−
F5074	Seed R6	13.5	n.d.	n.d.	n.d.	28.6	−
F5083	Seed R6	n.d.	n.d.	n.d.	n.d.	3.0	+
F5161	Seed R6	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5094	Pod R8	16.5	n.d.	n.d.	n.d.	n.d.	−
F5158	Pod R8	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5168	Pod R8	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5175	Pod R8	n.d.	n.d.	n.d.	n.d.	11.0	+
F5023	Seed R8	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5160	Seed R8	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5164	Seed R8	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5166	Seed R8	n.d.	n.d.	n.d.	n.d.	n.d.	+
F5167	Seed R8	n.d.	n.d.	n.d.	n.d.	n.d.	−
F5170	Seed R8	n.d.	n.d.	n.d.	n.d.	25.0	+
F5183	Seed R8	n.d.	n.d.	n.d.	n.d.	n.d.	−

Notes: n.d., Not detected.
LODs: 0.1 $\mu g\,g^{-1}$ for DAS and NEO; 0.2 $\mu g\,g^{-1}$ for T-2 and HT-2; 1 $\mu g\,g^{-1}$ for DON and NIV; and 2 $\mu g\,g^{-1}$ for ZEA.

Mycotoxin production

Strains were cultured in Erlenmeyer flasks (250 ml) containing 25 g of long-grain rice. A total of 10 ml of distilled water was added before autoclaving for 30 min at 121°C, twice. Each flask was inoculated with a 3 mm diameter agar disk taken from the margin of a colony grown on synthetic nutrient agar (SNA) at 25°C for seven days. Flasks were shaken one per day by hand for 1 week. These cultures were incubated for 28 days at 25°C in the dark. At the end of the incubation period the contents of the flask were dried at 50°C for 24 h and then stored at −20°C until analysed for toxin.

Mycotoxin extraction and clean-up

For the extraction of toxins, each sample was finely ground in a laboratory grinder and homogenised. A subsample of ground rice (15 g) was extracted by mixing with 100 ml of acetonitrile:water (84:16, v/v), shaken for 2 h on a oscillatory shaker and then filtered through Whatman No. 4 filter paper. Clean-up was carried out with a Mycosep™ 227 column (Romer Labs Inc., Union, MO, USA). The filtrate (8 ml) was transferred to a culture tube and slowly pressed into the interior of the tube with the rubber flange end turned down until 6 ml of the extract had passed through the column. Them, 1 ml of the purified extract was transferred to a vial and evaporated to dryness under nitrogen at 60°C.

Type A trichothecenes analysis by HPLC/FD and recovery experiments

The dried residue was derivatised as previously described for T-2 and HT-2 toxins by Visconti et al. (2005). A total of 50 µl of DMAP solution (0.325 µg µl^{-1} in toluene) followed by 50 µl of 1-AN reagent (0.3 µg µl^{-1} in toluene) were added to the dried residue. The vial was closed and mixed by vortex for 1 min. The mixture was left to react for 15 min at 50°C in a heater block and then cooled in ice for 10 min. The reaction mixture was dried under a gentle stream of nitrogen at 50°C and reconstituted with 1000 µl of an acetonitrile/water (70:30, v/v) mixture and homogenised in a vortex mixer for 30 s. A total of 50 µl of the solution were injected into the HPLC system by full-loop injection technique (Hewlett Packard model 1100 pump; Hewlett Packard, Palo Alto, CA, USA; and Rheodyne manual injector with a 50 µl loop; Rheodyne, Cotati, CA, USA). Chromatographic separations were performed on a Phenyl-Hexyl Luna® column (150 mm × 4.6 mm i.d., 5 µm particle size; Luna-Phenomenex, Torrance, CA, USA) connected to a pre-column Security Guard (20 mm × 4.6 mm i.d., 5 µm particle size; Phenomenex). The flow rate of the mobile phase was 1.0 ml min^{-1}. A binary gradient was applied as follows: the initial composition of the mobile phase, 70% acetonitrile/30% water, was kept constant for 5 min and then the acetonitrile content was linearly increased to 85% in 10 min, and kept constant for 10 min. Finally, to clean the column the amount of acetonitrile was increased to 100% in 2 min and kept constant for 5 min. The mycotoxins were detected by fluorometric detection (Hewlett Packard model 1046A programmable fluorescence detector) at 381 nm (excitation wavelength) and 470 nm (emission wavelength) and quantified by a data module Hewlett Packard Kayak XA (HP ChemStation Rev. A.06.01).

DAS, NEO, T-2 and HT-2 stock solutions (1 mg ml^{-1} each) were prepared by dissolving DAS, NEO, T-2 and HT-2 solid commercial toxins (Sigma-Aldrich, Milan, Italy) in acetonitrile (HPLC grade). A mixed DAS, NEO, T-2 and HT-2 standard solution (2 µg ml^{-1} each mycotoxin) for spiking purposes was prepared by diluting adequate amounts of the stock solutions. Standard solutions for calibration curves were prepared by diluting aliquots of the mixed DAS, NEO, T-2 and HT-2 standard solution in acetonitrile. Aliquots of mycotoxin standard solutions (corresponding to 40–200 ng of each toxin) were placed in screw-cap amber vials, and the solvent was evaporated to dryness at about 50°C under a stream of air before derivatisation. Mycotoxin levels were calculated by comparing the area of the chromatographic peaks relevant to DAS, NEO, T-2 and HT-2 in the purified samples with those of the standard calibration curves.

Recovery experiments for type A trichothecenes were performed in triplicate by spiking blank rice samples with DAS, NEO T-2 and HT-2 at levels of 0.5 and 1.0 µg g^{-1}. Spiked samples were left for 1 h to allow solvent evaporation prior to extraction.

Detection limits were 0.1 µg g^{-1} for DAS and NEO and 0.2 µg g^{-1} for T-2 and HT-2 toxins.

Type B trichothecenes analysis by HPLC/UV

For the detection of NIV and DON, the analysis was performed using the method described by Barros et al. (2008). The dried residue obtained after Mycosep™ 227 column clean-up was dissolved in 400 µl of methanol/water (5:95, v/v), homogenised in a vortex mixer and injected into the HPLC system by a full-loop injection technique (Hewlett Packard model 1100 pump; Rheodyne manual injector with a 50 µl loop). The HPLC system consisted of a Hewlett Packard model 1100 pump connected to a Hewlett Packard 1100 Series variable wavelength detector and a data module Hewlett Packard Kayak XA (HP ChemStation Rev. A.06.01). Chromatographic separations were performed on a Luna™ C18 reversed-phase column (100 × 4.6 mm, 5 µm particle size) connected to a guard column SecurityGuard™ (4 × 3.0 mm) filled with the

same phase. The mobile phase consisted of methanol/water (12:88, v/v), at a flow rate of 1.5 ml min^{-1}. The detector was set at 220 nm with an attenuation of 0.01 AUFS. Quantification was relative to external standards of DON and NIV (Sigma-Aldrich Co., St Louis, MO, USA) of 1–4 μg ml^{-1} in methanol/water (5:95). The detection limit was 1 μg g^{-1} for each toxin.

Zearalenone analysis

The production of ZEA was tested by thin-layer chromatography (TLC). Each sample was finely ground in a laboratory grinder and homogenised. A subsample of ground rice (15 g) was extracted by mixing with 40 ml acetonitrile/methanol (14:1, v/v), shaken for 2 h on a oscillatory shaker and then filtered through Whatman No. 4 filter paper. A syringe (3 ml capacity) plugged with glass wool and dry-packed with alumina/carbon (20:1, w/w; 500 mg) was used as a mini-clean-up column. A 2 ml aliquot of extract was applied to the column and allowed to drain under gravity. The eluate was collected in a 4 ml glass vial. The column was then washed with 500 μl acetonitrile/methanol/water (80:5:15), and the combined eluates evaporated to dryness under N_2 at 50°C. The residues were dissolved in 100 μl of chloroform and 5, 10 and 15 μl of each extract were placed on to the silica gel plate and developed in chloroform/acetone (9:1, v/v) using ZEA (Sigma-Aldrich) as an external standard. Developed TLC plates were examined under long-wave UV light at 360 nm. For the confirmation of positive samples, spray reagent of *bis*-diazotised benzidine was applied (Malaiyandi et al. 1976). The detection limit was 2 μg g^{-1}.

Results and discussion

F. equiseti and *F. semitectum* represent the most abundant species in the *Fusarium* complex isolated from flowers, soybean pods and seeds in Argentina (Broggi et al. 2007; Barros et al. 2012). Although these fungi are not considered to be a component of the *Fusarium* head blight disease complex (Gale 2003; Logrieco et al. 2003; Shaner 2003) there is a lack of information on the toxigenic potential among strains of these species isolated from the soybean agroecosystem.

The production of type A trichothecenes among the *F. equiseti* and *F. semitectum* isolates was evaluated by HPLC method with fluorescence detection (FD) using 1-anthronylnitrile as the derivatising reagent. Results of the recovery experiments showed that within the spiking range of 0.5 and 1.0 μg g^{-1}, mean recoveries were 84.0%, 76.8%, 75.5% and 74.1% for DAS, NEO, T-2 and HT-2, respectively, with a within-laboratory relative standard deviation (RSDr) $<3\%$. A similar method using coumarin-3-carbonyl chloride as a derivatising reagent was used by Mateo et al. (2002) to determine the type A trichothecenes produced by *F. sporotrichioides* in maize, wheat and rice cultures. Figure 1 shows the chromatogram of combined standard solution of the four type A trichothecenes evaluated (DAS, NEO, T-2 and HT-2).

None of the isolates of *F. semitectum* produced detectable levels of DAS, NEO, T-2 and HT-2 under the given conditions. In relation to *F. equiseti*, only DAS production was observed by 15% of the isolates tested with levels within a range of 0.4–16.5 μg g^{-1} (median of 2 μg g^{-1}). Figure 2 shows a well-resolved chromatogram of a DAS-producing strain without interfering peaks from the rice culture. Kosiak et al. (2005)

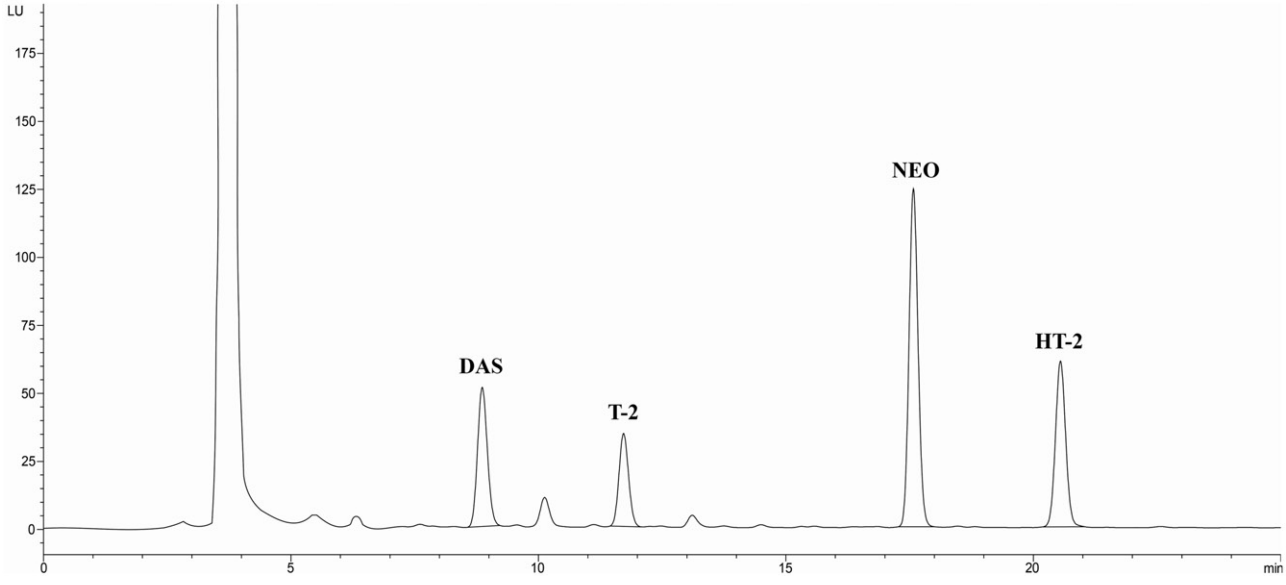

Figure 1. Chromatogram of a standard solution of DAS, T-2, NEO and HT-2 after derivatisation with 1-AN.

evaluated the production of trichothecenes in 27 strains of *F. equiseti* isolated from wheat, oats and barley in Norway. The present study showed that the total strains tested were DAS producers; however, the levels detected were lower than those in the present study.

Regarding NEO production, none of the soybean agroecosystem isolates produced detectable levels of this mycotoxin. This result coincides with that of Hestbjerg et al. (2002) who tested the production of this secondary metabolite in 57 *F. equiseti* isolates in different conditions and culture media. In contrast, Kosiak et al. (2005) included NEO in the profile of Norwegian strains of *F. equiseti*, since 16 of 27 tested isolates produced NEO.

A previous survey showed a low incidences and levels of T-2 and HT-2 toxins in soybean harvested among the areas in Cordoba Province, Argentina (Barros et al. 2011). In this study none of the strains of *F. equiseti* in the same area produced detectable levels of these toxins. This would demonstrate the fact that *F. equiseti* is not associated with such contamination, despite being the predominant *Fusarium* species in all reproductive stages of soybean cultivation to maturity. No production of T-2 and HT-2 toxins is consistent with the results found by Kosiak et al. (2005).

Regarding type B trichothecenes, only one of 22 *F. semitectum* isolates produced NIV at a level close to the detection limit. In *F. equiseti*, 13 of 40 isolates (33%) produced NIV levels in a range from 2.5 to 93.0 $\mu g\,g^{-1}$ (median of 16 $\mu g\,g^{-1}$) and none of the strains showed production of DON. A recent study has determined the natural occurrence of NIV in wheat in Argentina (Palacios et al. 2010). Considering that *F. equiseti* was the predominant species in the contaminated wheat at harvest time and taking into account that the soybean crop is planted regularly in rotation with wheat, it would be important to determine the potential contribution of *F. equiseti* to such contamination. Hestbjerg et al. (2002) suggested that the production of type B trichothecenes by *F. equiseti* seems rather controversial. They found that seven of eight trichothecenes-producing isolates produced one or more metabolites within the type B trichothecenes.

The production of trichothecenes in some *Fusarium* species has been related to plant pathogenesis (Goswami and Kistler 2005). In a previous study, the highly conserved production of DON and its acetylated derivatives among the members *F. graminearum* complex isolated from soybean agroecosystem was demonstrated (Barros et al. 2012). In contrast, the trichothecene production by *F. equiseti* seems to be variable among the isolates tested. This may indicate a low selection pressure for this trait in *F. equiseti* as was suggested by Hestbjerg et al. (2002).

In relation to ZEA production, the evaluation of this toxin was done using a qualitative analytical method (TLC), so the results are reported as production/no production. On this basis, it was observed that only one *F. semitectum* isolate produced ZEA, while 35% of the *F. equiseti* isolates produced the mycotoxin under the conditions evaluated. This is consistent with previous studies that reported the production of this mycotoxin by *F. equiseti* (Langseth et al. 1999; Hestbjerg et al. 2002; Logrieco et al. 2003; Kosiak et al. 2005).

In conclusion, nearly 50% of *F. equiseti* isolates produce some type A and type B trichothecene (i.e. DAS and NIV) and/or ZEA, whereas only two

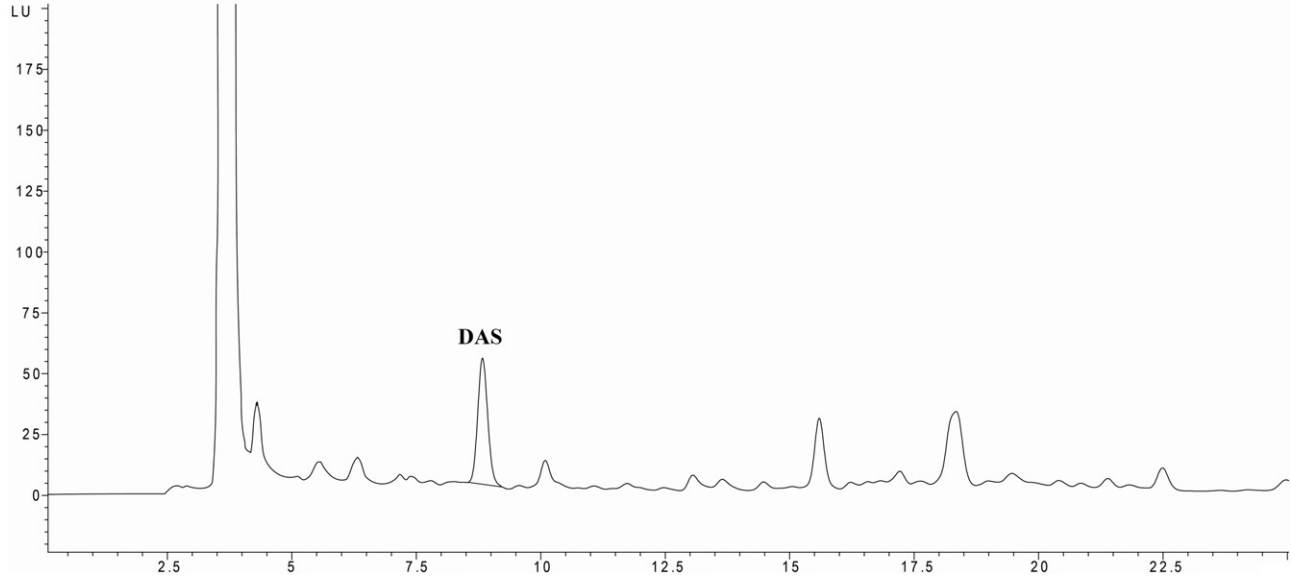

Figure 2. Chromatogram of a rice culture extract of *F. equiseti* isolate F5094 after Mycosep^{TM} 227 column clean-up and derivatisation with 1-AN (DAS found 16.5 $\mu g\,g^{-1}$).

F. semitectum isolates produced detectable levels of these mycotoxins. Both *F. equiseti* and *F. semitectum* did not produce any DON, NEO, T-2 toxin and HT-2 toxin. The variable retention in toxigenicity displayed by both species would demonstrate that they could have a saprophytic lifestyle in the soybean agroecosystem in Argentina.

Acknowledgements

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References

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.

Barros G, García D, Oviedo S, Ramirez L, Torres A, Chulze S. 2008. Deoxynivalenol and nivalenol analysis in soybean and soy flour. World Mycot J. 1(3):263–266.

Barros G, García D, Oviedo S, Ramirez ML, Torres A, Lattanzio V, Pascale M, Chulze S. 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 Mycot J. 4(2):189–197.

Berthiller F, Schumacher R, Buttinger G, Krska R. 2005. Rapid simultaneous determination of major type A- and B-trichothecenes as well as zearalenone in maize by high performance liquid chromatography-tandem mass spectrometry. J Chromatogr A. 1062:209–216.

Bosch U, Mirocha CJ. 1992. Toxin production by *Fusarium* species from sugar beets and natural occurrence of zearalenone in beets and beet fibers. Appl Environ Microbiol. 58:3233–3239.

Broggi L, González HHL, Resnik SL, Pacin AM. 2007. Alternaria alternata prevalence in cereal grains and soybean seeds from Entre Ríos, Argentina. Rev Iberoam Micol. 24:47–51.

Cohen H, Boutin-Muma B. 1992. Fluorescence detection of trichothecene mycotoxins as coumarin-3-carbonyl chloride derivatives by high performance liquid chromatography. J Chrom. 595:143–148.

Croteau SM, Prelusky DB, Trenholm HL. 1994. Analysis of trichothecene mycotoxins by gas chromatography with electron capture detection. J Agric Food Chem. 42:928–933.

Desjardins AE. 2006. Fusarium mycotoxins: chemistry, genetics, and biology. St. Paul (MN): APS Press.

Furlong EB, Valente-Soares LM. 1995. Gas chromatographic method for quantitation and confirmation of trichothecenes in wheat. J Assoc Off Anal Chem. 78:386–390.

Gale LR. 2003. Population biology of *Fusarium* species causing head blight of grain crops. In: Leonard KJ, Bushnell WR, editors. *Fusarium* head blight of wheat and barley. St. Paul (MN): The American Phytopathological Society. p. 120–143.

Goswami RS, Kistler CH. 2005. Pathogenicity and in planta mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. Phytopathology 95:1397–1404.

Hestbjerg H, Nielsen KF, Thrane U, Elmholt S. 2002. Production of trichothecenes and other secondary metabolites by *Fusarium culmorum* and *Fusarium equiseti* on common laboratory media and a soil organic matter agar: an ecological interpretation. J Agric Food Chem. 50:7593–7599.

Jiménez M, Mateo JJ, Mateo R. 2000. Determination of type A trichothecenes by high performance liquid chromatography with coumarin-3-carbonyl chloride derivatisation and fluorescence detection. J Chromatogr A. 870:473–481.

Kosiak EB, Holst-Jensen A, Rundberget T, Jaen MTG, Torp M. 2005. Morphological, chemical and molecular differentiation of *Fusarium equiseti* isolated from Norwegian cereals. Int J Food Microbiol. 2(99):195–206.

Krska R, Baumgartner S, Josephs R. 2001. The state-of-the-art in the analysis of type-A and -B trichothecene mycotoxins in cereals. Fresenius J Anal Chem. 371:285–299.

Langseth W, Bernhoft A, Rundberget T, Kosiak B, Gareis M. 1999. Mycotoxin production and cytotoxicity of *Fusarium* strains isolated from Norwegian cereals. Mycopathologia 144:103–113.

Lattanzio VMT, Solfrizzo M, Visconti A. 2007. Determination of trichothecenes in cereals and based cereal products by liquid chromatography-tandem mass spectrometry. Food Add Contam. 25:320–330.

Leslie JF, Summerell BA. 2006. The *Fusarium* laboratory manual. Ames (IA): Blackwell Professional.

Lippolis V, Pascale M, Maragos C, Visconti A. 2008. Improvement of detection sensitivity of T-2 and HT-2 toxins using different fluorescent labeling reagents by high-performance liquid chromatography. Talanta 74:1476–1483.

Logrieco A, Bottalico A, Mulé G, Moretti A, Perrone G. 2003. Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. Eur J Plant Pathol. 109:645–647.

Malaiyandi M, Barrette JP, Wavrock PL. 1976. Bis-diazotized benzidine as spray reagent for detecting zearalenone on thin layer chromatoplates. J Assoc Off Anal Chem. 59:959–962.

Marasas WFO, Nelson PE, Toussoun TA. 1984. Toxigenic *Fusarium* species: identity and mycotoxicology. University Park (PA): Pennsylvania State University Press.

Mateo J, Mateo R, Jiménez M. 2002. Accumulation of type A trichothecenes in maize, wheat and rice by *Fusarium sporotrichioides* isolates under diverse culture conditions. Int J Food Microbiol. 72:115–123.

Ministerio de Agricultura Ganadería Pesca y Alimentación de la Nación (MAGPyA). 2011. Soja Informe general; [cited 2011 May 27]. Available from: http://www.sagyp.mecon.gov.ar/new/0-0/agricultura/otros/estimaciones/soja/infsoja/php/

Nirenberg HI. 1976. Untersuchungen über die morphologische und biologisch Diffrenzieerum in der Fusarium Sekion Lisiola. Mitt Biol Bundenanst Land-Forstwirtsch. 169:1–117.

Palacios S, Farnochi MC, Ramirez ML, Reynoso MM, Zappacosta D, Soresi D, Torres A. 2010. Natural contamination with nivalenol and deoxynivalenol in durum wheat germoplasms in Argentina. In: Carvajal Moreno M, editor. Proceedings of the VIth Latin American Congress of Micotoxicology. Merida (Mexico): Sociedad Latinoamericana de Micotoxicología. p. 245–246.

Park JJ, Chu FS. 1993. Immunochemical analysis of trichothecenes produced by various fusaria. Mycopathologia 121:179–192.

Pascale M, Haidukowski M, Visconti A. 2003. Determination of T-2 toxin in cereal grains by liquid chromatography with fluorescence detection after immunoaffinity column clean-up and derivatization with 1-anthroylnitrile. J Chromatogr A. 989:257–264.

Pitt JI, Hocking AD. 1997. Fungi and food spoilage. Sydney (Australia): CSIRO Division of Food Science and Technology/Sydney Academic Press.

Shaner GE. 2003. Epidemiology of *Fusarium* head blight of small grain cereals in North America. In: Leonard KJ, Bushnell W, editors. *Fusarium* head blight of wheat and barley. St. Paul (MN): APS Press. p. 88–119.

Thrane U. 1989. *Fusarium* species and their specific profiles of secondary metabolites. In: Chelkowski J, editor. *Fusarium*: mycotoxins, taxonomy and pathogenicity. Amsterdam (the Netherlands): Elsevier. p. 199–226.

Thrane U. 2001. Developments in the taxonomy of *Fusarium* species based on secondary metabolites. In: Summerell BA, Leslie JF, Backhouse D, Bryden WL, Burgess LW, editors. *Fusarium*: Paul E. Nelson Memorial Symposium. St. Paul (MN): American Phytopathological Society. p. 29–49.

Visconti A, Lattanzio VMT, Pascale M, Haidukowski M. 2005. Analysis of T-2 and HT-2 toxins in cereal grains by immunoaffinity clean-up and liquid chromatography with fluorescence detection. J Chromatogr A. 1075: 151–158.

Wu WD, Nelson PE, Cook ME, Smalley EB. 1990. Fusarochromanone production by *Fusarium* isolates. Appl Environ Microbiol. 56:2989–2993.