JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Mycoflora and Mycotoxin Production in Oilseed Cakes during Farm Storage

CAROLINE LANIER,† NATACHA HEUTTE,† ESTELLE RICHARD,†
VALERIE BOUCHART,‡ PIERRE LEBAILLY,† AND DAVID GARON*,†

Groupe Régional d'Etudes sur le Cancer, GRECAN-EA 1772, IFR 146 ICORE, Université de Caen Basse-Normandie, Centre François Baclesse, avenue Général Harris, BP 5026, 14076 Caen Cedex 05, France, and Département Environnement-Alimentation, Laboratoire Départemental Frank Duncombe, 14280 Saint Contest, France

Agricultural activities involve the use of oilseed cakes as a source of proteins for livestock. Because the storage of oilseed cakes could induce the development of molds and the production of mycotoxins, a survey was conducted during the 5 months of farm storage. Mycoflora was studied by microscopic examinations, and the presence of *Aspergillus fumigatus* was confirmed by polymerase chain reaction. A multimycotoxin method was developed to quantify seven mycotoxins (aflatoxin B_1, alternariol, fumonisin B_1, gliotoxin, ochratoxin A, T-2 toxin, and zearalenone) in oilseed cakes by high-performance liquid chromatography coupled to mass spectrometry. Among 34 fungal species identified, *A. fumigatus* and *Aspergillus repens* were observed during 5 and 4 months, respectively. Gliotoxin, an immunosuppressive mycotoxin, was quantified in oilseed cakes up to 45 μg/kg, which was associated with the presence of toxigenic isolates of *A. fumigatus*.

KEYWORDS: Oilseed cakes; mycoflora; *Aspergillus fumigatus*; mycotoxins; gliotoxin; storage

INTRODUCTION

In cattle-breeding farms, oilseed cakes and corn silage represent the two most important feeds in France (*1*). Oilseed cakes, the solid residue obtained after seed pressing and oil extraction from oleaginous plants, constitute a valuable byproduct because they are used as an important source of proteins in animal nutrition. Oilseed cakes come from soya seeds [*Glycine max* (L.) Merr.], rape (*Brassica napus* L.), and sunflower (*Helianthus annuus* L.) and are often mixed in the form of pellets.

The seeds of these oleaginous plants could be contaminated by three major genera of fungi, that is, *Aspergillus*, *Fusarium*, and *Penicillium*. Although industrial processes of oil extraction require high heating (120 °C) and remove the greater part of mycotoxins, the storage of oilseed cakes could induce the activation of persistent spores and mold contamination by spores from air or other bordering feeds like corn silage and hay. Moisture and heat and attack from rodents or insects represent some factors that promote fungal invasion and/or proliferation, leading to the occurrence of mycotoxins (*2*). During storage, a large number of fungal species belonging to *Aspergillus*, *Fusarium*, *Monascus*, and *Penicillium* genera were previously reported in silages (*3*), but little is known about the fungal contamination and the mycotoxins production in oleaginous feeds during storage.

Mycotoxins are secondary metabolites produced by filamentous fungi. Some of these fungal toxins were known to be carcinogenic (aflatoxin B_1), nephrotoxic (ochratoxin A), or estrogenic (alternariol, zearalenone). For many mycotoxins (aflatoxin B_1, deoxynivalenol, fumonisin B_1, gliotoxin, ochratoxin A, T-2 toxin, or zearalenone), immune disorders were also observed during mycotoxicosis (*2*). Humans and animals are especially exposed to mycotoxins through contaminated food, but the inhalation of fungal spores is also possible. Fungi are involved in several pulmonary diseases like asthma, allergenic syndromes (*4*), or farmer's lung (*5*). Some data suggest the potential relation between aflatoxin B_1 in bioaerosols and the development of pulmonary cancers (*6*). In an agricultural environment, farmers could be exposed to spores and mycotoxins adsorbed on spores or dust during daily handling of vegetal matrices such as oilseed cakes. To explore the occupational exposure of farmers to fungi and mycotoxins, the first step consisted of obtaining environmental data about the contamination of oilseed cakes and required the coupling of analytical and microbiological tools.

The objectives of this work were to adapt a multimycotoxin analysis method for the detection of seven mycotoxins in oleaginous pellets and to survey and describe fungal contamination (i.e., mycoflora and mycotoxins) of oilseed cakes during their 5 months of farm storage.

* To whom correspondence should be addressed. Tel: (33)231 45 52 21. Fax: (33)231 45 51 72. E-mail: d.garon@baclesse.fr.
† Université de Caen Basse-Normandie.
‡ Laboratoire Départemental Frank Duncombe.

10.1021/jf8031588 CCC: $40.75
Published on Web 01/30/2009

Table 1. MS Conditions for Multimycotoxin Quantification in Oilseed Cakes

mycotoxins (toxin-producing species)	RT[a] (min)	mode (SIM)[b]	monitored ions (*m*/*z*)	abundance relative (%)	QL[c] (μg/kg)
aflatoxin B_1 (*Aspergillus flavus*, *Aspergillus parasiticus*)	15.9	+	313	QI[d]	40
		+	647	60	
		+	335	85	
alternariol (*Alternaria* spp.)	17.6	−	257	QI	20
		+	259	20	
		−	258	15	
fumonisin B_1 (*Fusarium verticillioides*)	18.8	+	722	QI	20
		+	723	35	
		+	724	10	
gliotoxin (*Aspergillus fumigatus*)	13.2	+	263	QI	10
		+	245	55	
		+	227	20	
ochratoxin A (*Aspergillus ochraceus*, *Penicillium verrucosum*)	23.6	+	404	QI	20
		+	406	35	
		+	405	20	
T-2 toxin (*Fusarium culmorum*, *Fusarium graminearum*)	22.5	+	489	QI	100
		+	484	16	
		+	305	25	
zearalenone (*Fusarium culmorum*, *Fusarium graminearum*)	23.6	−	317	QI	40
		−	318	18	

[a] Retention time. [b] SIM with positive (+) or negative (−) mode of ionization. [c] Quantification limit. [d] Quantification ion.

MATERIALS AND METHODS

Sample Collection. Pellets consisted of an oleaginous mixture of 70% rapeseeds, 15% soybeans, and 15% sunflower seeds. Samples were collected on a farm located in Calvados (Normandy, France). The selected farm was a dairy farm characterized by 372 acres of cultivated land and a total of 205 cattle mainly fed by corn silage and oilseed cakes.

Ten samples were taken each month with a silo-picker (VWR, Fontenay sous Bois, France) from the front of the pile. The monitoring was conducted in oilseed cakes stored for 5 months (September to January) in the farm. Each sample of 80 g was homogenized in a blender, and 10 g was randomly selected for the analysis of mycoflora. The other part (70 g) was stored at −20 °C before the multimycotoxin analysis.

Chemicals and Reagents. Seven mycotoxins (aflatoxin B_1, alternariol, fumonisin B_1, gliotoxin, ochratoxin A, T-2 toxin, and zearalenone), which could be possibly produced in oleaginous plants, were selected after a literature review. Standards were supplied by Sigma-Aldrich (St. Louis, MO). Stock solutions of 250 μg/mL were prepared in liquid chromatography (LC) grade methanol from Chromanorm VWR Prolabo (Fontenay sous Bois, France) and stored at −20 °C in the dark. Diluted solutions were prepared by diluting the stock solutions with mobile phase acetonitrile/water (10:90, v/v). The working solution, composed of the seven mycotoxins, was prepared by combining suitable aliquots of each individual standard stock dilution to obtain each mycotoxin at 0.5 μg/mL.

Milli-Q quality water (Millipore, Bedford, MA) and all other chemicals of LC grade were obtained from Prolabo (Fontenay sous Bois, France). Purification assays used Oasis HLB (6 mL, 200 mg) cartridges purchased from Waters (Milford, MA).

Extraction and Solid-Phase Cleanup of Mycotoxins from Oilseed Cakes. A portion of 5 g of oilseed cakes was weighed in an Erlenmeyer flask. Mycotoxins were extracted with 100 mL of a mixture of methanol/water (80:20, v/v) acidified with acetic acid (0.5%, pH 3), except for T-2 toxin. The slurry was ground by an Ultra-Turrax basic T25 homogenizer (IKA-Werke, Staufen, Germany), then shaken on a rotary shaker for 60 min at 100 rpm, and finally centrifuged at 302*g* for 30 min at 10 °C.

A volume of 10 mL of the supernatant obtained from the previous centrifugation was diluted in 90 mL of Milli-Q water and was then purified through an Oasis HLB cartridge, previously conditioned with 4 mL of methanol and equilibrated with 4 mL of Milli-Q water, at a flow rate of 1 mL/min. The cartridge was washed with 2 mL of Milli-Q water. Aflatoxin B_1, alternariol, fumonisin B_1, ochratoxin A, T-2 toxin, and zearalenone were eluted with 10 mL of a mixture of methyl *tert*-butyl ether (MTBE)/methanol (90:10, v/v) followed with 5 mL of methanol; gliotoxin was eluted with 10 mL of a mixture of MTBE/methanol (90:10, v/v). For recovery studies, oilseed cakes were fortified at 5, 10, 20, 40, 100, 200, 400, and 1000 μg/kg before extraction and purification as described above.

Multimycotoxin Detection by High-Performance Liquid Chromatography−Mass Spectrometry (HPLC-MS). The eluted mycotoxins were evaporated under vacuum in a Syncore polyvap parallel evaporator (Büchi Labotechnik AG, Flawil, Switzerland) and then finished to dryness under a stream of nitrogen. The final residue was dissolved in 1 mL of a mixture of acetonitrile/water (10:90, v/v). This was filtered through Millex HV 0.45 μm and injected into the HPLC-MS. LC was performed using an Agilent Technologies series 1100 (Palo Alto, CA) quaternary pump coupled with an autosampler and a model SL mass spectrometry detector. The analytes were chromatographed at 40 °C on a 150 mm × 2.1 mm i.d., 5 μm, Zorbax SB-C_{18} column (Agilent Technologies) with a 15 mm × 1 mm packed bed Optiguard C_{18} precolumn. Mycotoxins were separated using an elution gradient with water acidified with 0.5% acetic acid (pH 3) (solvent A) and acetonitrile (solvent B). The gradient program was as follows: at time zero, 95% solvent A; to 80% solvent A within 3 min; to 70% solvent A in 11 min; and to 50% solvent A in 6 min; and finally, to 30% solvent A in 7 min. The flow rate was 400 μL/min. The injection volume was 10 μL.

Mass spectrometry was performed on a quadrupole analyzer (Agilent Technologies) equipped with an electron spray ionization (ESI) source and operating in positive and negative modes. The parameters used for the mass spectrometer in all experiments were as follows: capillary voltage, 3.0 kV; solvent gas, 720 L/h; evaporation temperature, 350 °C; and pressure of nebulization, 35 psi. The ions monitored are presented in **Table 1**. Full scan mass spectra were recorded to select the most abundant *m*/*z* value, and then, selected ion monitoring (SIM) mode was used for the quantification.

Linearity was established by injecting increasing concentrations from eight extracted ranges (fortified at 5, 10, 20, 40, 100, 200, 400, and 1000 μg/kg). Quantification limits were determined by spiked samples based on a signal-to-noise ratio of 10:1.

Mycoflora of Oilseed Cakes. For each sample, two culture methods were applied. On the one hand, pellets of oilseed cakes were directly dropped on a Petri dish (90 mm diameter) containing malt extract (1.5%)/agar (1.5%) medium (MEA) complemented with chloramphenicol (0.05%, w/v); on the other hand, 5 g of oilseed cake was suspended in 20 mL of sterile water containing Tween 80 (0.05%, w/v). After 1 h of orbital shaking (240 rpm), 30 μL of each suspension was sprayed in a Petri dish (triplicate). The plates were incubated at 22, 30, and 37 °C. The identity of each strain, isolated and purified, was achieved through macro- and microscopic examinations after 7 and 14 days of culture (*7, 8*). For the species belonging to the *Penicillium* genus, growth was also observed on two selective media, Czapek yeast autolysate (CYA) agar and 25% glycerol nitrate agar (G25N), and incubated at 5, 22, and 37 °C. *Fusarium* species were cultured on MEA and potato dextrose agar (PDA) medium. All of the purified strains were preserved on agar slants (MEA) at 4 °C.

Detection of *Aspergillus fumigatus* by Polymerase Chain Reaction (PCR). Because *A. fumigatus* is an important opportunistic pathogen and a major cause of respiratory allergy, its presence in oilseed cakes was also confirmed by PCR. DNA was extracted from culture plates using Nucleospin Plant (Macherey Nagel, Düren, Germany). Mycelium and conidia were collected on each Petri dish and were incubated in 4 mL of absolute ethanol (Chromanorm VWR Prolabo) at 22 °C for 12 h. The conidia and mycelia suspension was centrifuged for 7 min at 3600*g* at 17 °C. Fungi cells were lysed into Qiagen TissueLyser (Valencia, CA) with glass beads and 250 μL of lysis buffer for 15 min at 30 Hz, and then, 400 μL of chloroform (Sigma-Aldrich) and 20 μL of proteinase K at 10 mg/mL (Sigma-Aldrich) were added. The aqueous supernatant was recovered after a centrifugation for 5 min at 20800*g* and was filtered on Nucleospin column after incubation for 30 min at 60 °C. DNA was precipitated in 600 μL of buffer and 400 μL of absolute ethanol. The pellet was washed using various washing buffers supplied by the manufacturer. Elution was performed with 2 × 50 μL elution buffer preincubated at 70 °C.

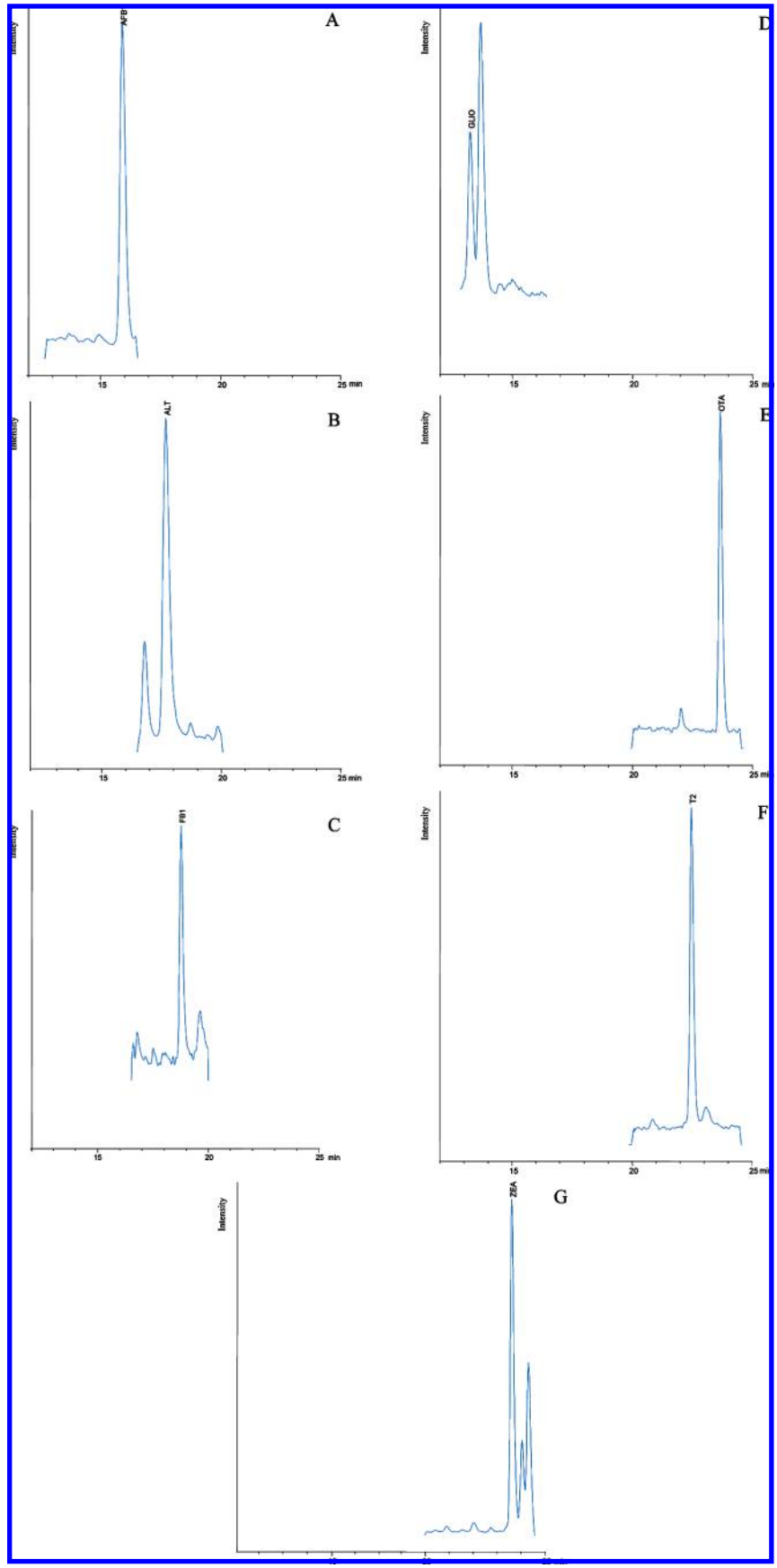

Figure 1. Chromatograms of the seven mycotoxins in fortified extracts of oilseed cakes (150 μg/kg). (**A**) AFB1, aflatoxin B_1; (**B**) ALT, alternariol; (**C**) FB_1, fumonisin B_1; (**D**) GLIO, gliotoxin; (**E**) OTA, ochratoxin A; (**F**) T2, T-2 toxin; and (**G**) ZEA, zearalenone.

The primers Af measured 401 pb (forward primer, 5′-CCTTGG-TAGATTTGTTGGC-3′ and reverse primer, 5′-TCAACCGACTC-CCCTCAACC-3′), which amplified a DNA fragment of the 26S/ intergenic spacer region of the rDNA complex of *A. fumigatus* (*9*). PCR reactions were carried out in 50 μL volumes containing 100 ng of template DNA, 0.5 μM primers (Eurogentec, Seraing, Belgium),

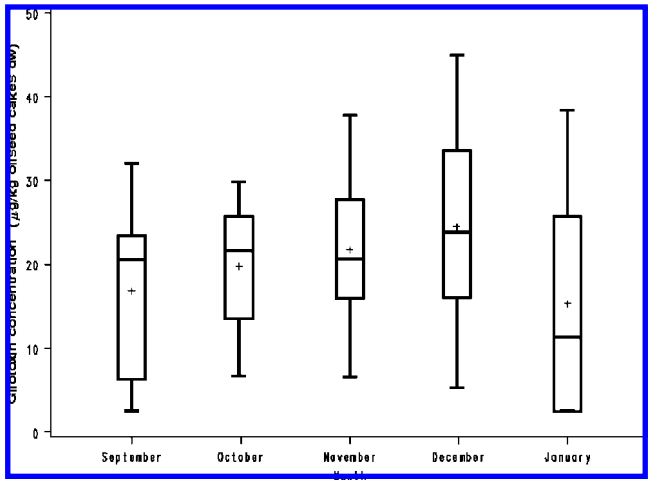

Figure 2. Box-and-whisker plots of monthly gliotoxin concentrations. Boxes range from the 25th to the 75th percentile; a horizontal line inside the box represents the median. Whiskers represent the minimum and the maximum values. The plus sign inside the box represents the mean.

Table 2. Statistical Analysis of Gliotoxin Concentrations in Oilseed Cakes

month	N[a]	gliotoxin concentration (µg/kg oilseed cake dw)[b]	P value of Shapiro−Wilk test[c]	P value of Bartlett test[d]	P value of ANOVA test
September	8	16.85 ± 10.35	0.25	0.43	0.33
October	10	19.80 ± 7.33	0.78		
November	10	21.75 ± 9.29	0.95		
December	10	24.48 ± 11.73	0.99		
January	6	15.33 ± 13.89	0.07		

[a] Number of contaminated samples. [b] Mean and standard deviation. [c] Test of normality sample. [d] Test of variance comparison.

2.5 U/reaction of *Taq* polymerase (Roche for Applied Biosystems, NJ), 5 µL of 1× PCR buffer (Roche for Applied Biosystems) supplemented with 1.5 mM $MgCl_2$ (Roche for Applied Biosystems) and 5% glycerol (Sigma-Aldrich), and 0.25 mM deoxynucleoside triphosphates (Applied Biosystems, Warrington, United Kingdom).

PCR for amplification of *A. fumigatus* DNA fragment was carried out on an programmable DNA thermal cycler (Mastercycler Gradient Eppendorf, Westbury, NY) programmed for 1 cycle of 10 min at 94 °C and 31 cycles of 1 min at 94 °C followed by 1 min 20 at 56 °C and 2 min at 72 °C. A 5 min final extension was added at 72 °C. The PCR products were analyzed by electrophoresis on 1% agarose gel in 1× TBE (Sigma-Aldrich) stained with 0.30 µg of ethidium bromide per mL.

Gliotoxinogenic Ability of *A. fumigatus* Isolates. Forty-two isolates of *A. fumigatus* were purified and tested *in vitro* for gliotoxin production (triplicate). After 2 weeks of culture on MEA, for each dish, three agar plugs were extracted by ethyle acetate acidified with 1% acetic acid, and gliotoxin was quantified by HPLC-MS as described previously (*10*).

Statistical Analysis. Statistical analyses were performed for each detected mycotoxin. Normality on each sample was examined by Shapiro-Wilk test, and equality of variances was assessed by Bartlett test. Analysis of variance (ANOVA) was used to compare mean concentrations of gliotoxin in oilseed cakes. A nonparametric ANOVA (Kruskal−Wallis test) was used to compare median concentrations of gliotoxin produced by *A. fumigatus*.

SAS 9.1 software was used to analyze the data. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Multiresidue Method Adapted to Oilseed Cakes. A first protocol based on a previous method used in corn silage (*3*) was tested. Because the recoveries did not exceed 29% except for alternariol (54%) and T-2 toxin (57%), an optimization based on acidification of the extraction solvent (pH 3) and double elution during the cleanup step allowed us to obtain the same protocol for the survey of seven mycotoxins. **Figure 1** shows the chromatograms of the seven mycotoxins quantified in HPLC-MS. Final recoveries of the optimized method were 65 (gliotoxin), 70 (ochratoxin A, zearalenone), 76 (aflatoxin B_1), 85 (alternariol, fumonisin B_1), and 92% (T-2 toxin). The quantification limits were presented in **Table 1**. Calibration curves were linear in the studied range, showing correlation coefficients greater than 0.99.

Multimycotoxin Monitoring of Oilseed Cakes. Among the seven mycotoxins, only gliotoxin was quantified at concentrations between 5 and 45 µg/kg. **Figure 2** shows the monthly

Table 3. Mycoflora and Number of Contaminated Samples from Oilseed Cakes[a]

fungal strains	September	October	November	December	January
total no. of samples	10	10	10	10	10
Absidia corymbifera					*2*
Absidia cylindrospora	*1*		*2*	*1*	*4*
Acremonium fusidioides	*1*				
Acremonium roseum	*1*				
Alternaria alternata	*5*	*1*	*2*	*1*	
Alternaria tenuissima	*1*				
Aspergillus alliaceus	***1***				
Aspergillus flavus		***1***			
Aspergillus fumigatus[b]	***9***	***9***	***9***	***7***	***10***
Aspergillus niger		***2***			
Aspergillus parasiticus	***3***			***1***	
Aspergillus repens	***4***	***1***	***2***	***9***	***9***
Aspergillus versicolor					***1***
Cladosporium cladosporioides	*7*	*2*	*3*	*3*	*3*
Epicoccum purpurascens	*2*				
Eurotium amstelodami	***1***			***3***	
Eurotium chevalieri	***3***		***1***		
Eurotium herbariorum	***2***	***1***	***2***	***1***	
Fusarium graminearum	*2*	*1*			
Geotrichum candidum				*1*	*1*
Monascus ruber	*1*		*2*	*2*	*1*
Mucor hiemalis		*2*	*3*	*1*	*1*
Penicillium aurantiogriseum	*1*				
Penicillium chrysogenum	*3*	*1*	*1*	*5*	
Penicillium implicatum				*1*	
Penicillium restrictum	*1*				
Penicillium roqueforti	*1*				
Periconia glyceriicola	*1*				
Rhizomucor pusillus	*1*				
Scopulariopsis brevicaulis	*8*	*3*	*4*	*2*	*6*
Thermomyces lanuginosus	*1*				
Trichoderma viride		*1*	*1*		*1*
Trichothecium roseum		*1*			
Ulocladium chartarum	*2*	*1*	*2*	*5*	

[a] The presence of fungal species and the number of contaminated samples are indicated as italic. *Aspergillus* and *Eurotium* are indicated in bold. [b] All samples contaminated by *A. fumigatus* were found positive in PCR.

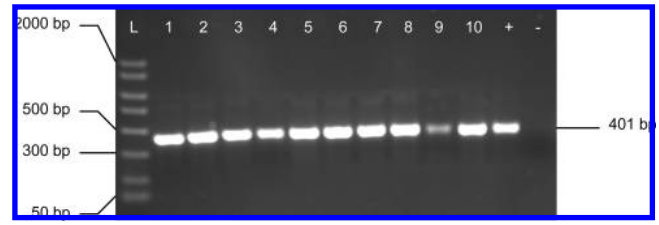

Figure 3. Gel electrophoretic analysis of PCR products using Af primers and DNA obtained from extracts of oilseed cakes collected in January. L, 50 bp ladder; lanes 1−10, number in sample collection collected in January; +, positive control; and −, negative control.

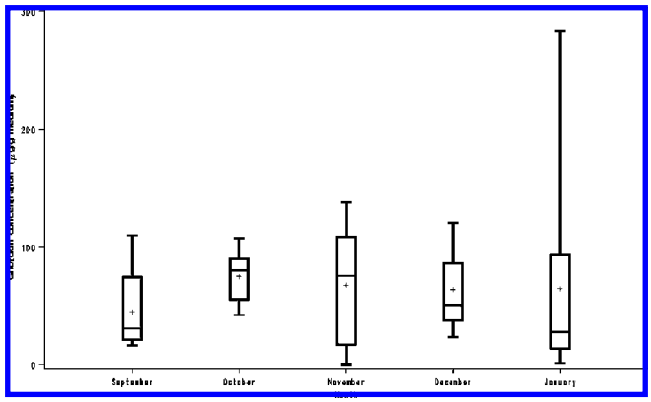

Figure 4. Box-and-whisker plots of gliotoxin production by monthly *A. fumigatus* isolates. Boxes range from the 25th to the 75th percentile; a horizontal line inside the box represents the median. Whiskers represent the minimum and the maximum values. The plus sign inside the box represents the mean.

concentration of gliotoxin in oilseed cakes. The contamination was observed since the first month of monitoring. The statistical analysis (**Table 2**) revealed that the mean concentration of gliotoxin did not significantly vary from September to January. Because gliotoxin is not detected during the industrial process, the production seems to have occurred in the first weeks of storage and to have remained stable during storage. Gliotoxin is one of the most abundantly produced epithiodioxopiperazine metabolites from *A. fumigatus*. Toxicological studies showed that gliotoxin can exacerbate the pathogenesis of aspergillosis (*11*), and low concentrations of gliotoxin are able to inhibit the activation of NF-κB, a central mediator of the immune response (*12*). *A. fumigatus* is also capable of producing several other secondary metabolites such as the tremorgenic fumitremorgens, the immunotoxic fumigaclavine C (*13*), and verruculogen, a neurotoxic compound able to modify electrophysiological properties of human nasal epithelial cells (*14*). Although *A. fumigatus* and/or gliotoxin have been detected in various forages (*10*, *15*), there is little data about the exposure to *A. fumigatus* toxins during the handling of contaminated vegetal matrices in agricultural areas. Moreover, toxins of *A. fumigatus* were relatively stable during storage (*16*), which was confirmed by the nonsignificant difference in gliotoxin concentrations during the 5 months of monitoring observed in our study. In further studies, other mycotoxins like verruculogen or fumitremorgens produced by *A. fumigatus* should be searched to complete the profile of mycotoxins in oilseed cakes.

Our study also revealed traces of alternariol in two samples of oilseed cakes (17 and 19 μg/kg) between the detection and the quantification limits (10 and 20 μg/kg, respectively). Alternariol belongs to mycotoxins produced by *Alternaria* species. This mycotoxin showed estrogenic and clastogenic effects in mammalian cells (*17*).

Previous studies showed that other mycotoxins could be produced in oleaginous plants, including aflatoxins in soyabean (*18*); alternariol in sunflower and colza (*19*); T-2 toxin, HT2, and zearalenone in sunflower seeds (*20*); fumonisins in peanut (*21*); and ochratoxin A in peanut and soyabean (*22*).

Mycoflora of Oilseed Cakes and Toxigenicity of *A. fumigatus* Isolates. The results from the monitoring of the oilseed cakes mycoflora during the 5 months of storage are given in **Table 3**. This investigation allowed the identification of 34 fungal species with seven species of *Aspergillus* and three species of *Eurotium*, respectively. The fungal diversity decreases from September to January. *A. fumigatus* and *Aspergillus repens* were recurrent during the monitoring and became the two predominant species in the fifth month.

The presence of gliotoxin was associated with the identification of *A. fumigatus* by microscopic examinations and the confirmation by PCR technology during the 5 months of monitoring. **Figure 3** presents the electrophoretic gel obtained with the samples collected in January. Among the 42 isolates of *A. fumigatus* tested *in vitro*, 41 strains were able to produce gliotoxin on nutrient agar up to 283 μg/g (**Figure 4**). No statistical differences were observed between median concentrations of gliotoxin during the 5 months ($p = 0.04$).

A. fumigatus was previously observed in corn silage (*3*, *10*). *A. repens*, distinguished by its osmophilic properties, forms part of the *Aspergillus glaucus* group. Recent information about toxinogenesis is not available for this group. *Eurotium amstelodami*, *Eurotium herbariorum*, and *Eurotium chevalieri* belong to this group and were identified during the monitoring. Among *Aspergillus* genus related to section *Flavi*, we noted the presence of *Aspergillus flavus* and *Aspergillus parasiticus*, frequently isolated as storage molds in cereals and oilseed product, and the ochratoxin A producer, *Aspergillus alliaceus*, previously isolated from nuts and figs (*23*). *Aspergillus niger* and *Aspergillus versicolor* were also identified in oilseed cakes; however, related mycotoxins (i.e., ochratoxin A and aflatoxins) were not detected by HPLC-MS. In spite of the presence of several potential toxigenic species in oilseed cakes, it would seem that the storage conditions are only favorable to gliotoxin production by *A. fumigatus*.

During the survey, the genera *Alternaria* and *Cladosporium*, known as allergenic fungi, remained in oilseed cakes for 4 and 5 months, respectively (*24*). *Alternaria* occurs in several food crops and may produce mycotoxins like tenuazonic acid and alternariol (*25*), whereas *Ulocladium* is a major allergen but its ability to produce mycotoxin seems not fully understood. *Scopulariopsis brevicaulis*, identified every month in oilseed cakes, is frequently involved in respiratory allergy (*4*). Other fungi are responsible for respiratory diseases such as *Alternaria* spp. in asthma (*26*), *E. amstelodami* in farmer's lung disease (*27*), and *A. glaucus* in hypersensitivity pneumonitis (*28*).

In conclusion, storage is the most critical postharvest phase in feed handling. Inappropriate storage can cause fungal development and mycotoxin production. Before studying exposure of farmers to fungi and mycotoxins during their occupational activities, the nature and source of mycotoxin contamination should be identified and described by combining an analytical method with the multimycotoxin quantification and microbiological tools with the monitoring of mycoflora.

A. fumigatus was previously detected in domestic and occupational environments like biowaste-handling facilities (*29*). Recently, a gardener developed a fatal aspergillosis and died after inhalation of decayed plant matter contaminated with *A. fumigatus* spores (*30*). The presence of toxigenic isolates of *A. fumigatus* and gliotoxin in oilseed cakes demonstrates that *A. fumigatus* and its related highly toxic metabolites like gliotoxin should be monitored during agricultural activities.

ACKNOWLEDGMENT

We thank R. Picquet and Y. Lecluse for their technical assistance.

LITERATURE CITED

(1) AGRESTE, 2004. Statistiques Agricoles, Ministère de l'Agriculture et de la Pêche; http://www.agreste.agriculture.gouv.fr/ (accessed September 15, 2008).

(2) Bennett, J. W.; Klich, M. Mycotoxins. *Clin. Microbiol. Rev.* **2003**, *16*, 497–516.
(3) Garon, D.; Richard, E.; Sage, L.; Bouchart, V.; Pottier, D.; Lebailly, P. Mycoflora and multimycotoxin detection in corn silage: Experimental study. *J. Agric. Food Chem.* **2006**, *54*, 3479–3484.
(4) Kurup, V. P.; Shen, H. D.; Banerjee, B. Respiratory fungal allergy. *Microb. Infect.* **2000**, *2*, 1101–1110.
(5) Roussel, S.; Reboux, G.; Dalphin, J. C.; Bardonnet, K.; Millon, L.; Piarroux, R. Microbiological evolution of hay and relapse in patients with farmer's lung. *Occup. Environ. Med.* **2004**, *61*, e3.
(6) Dvořáčková, I. Aflatoxin inhalation and alveolar cell carcinoma. *Br. Med. J.* **1976**, 6011–691.
(7) Samson, R. A.; Hoekstra, E. S.; Frisvad, J. C.; Filtenborg, O. *Introduction to Food and Airborne Fungi*; Centraalbureau voor Schimmelcultures: Utrecht, The Netherlands, 2002; 389 pp.
(8) Von Arx, J. A. *The Genus of Fungi Sporulating in Pure Culture*; Cramer: Vaduz, Germany, 1981; 424 pp.
(9) Spreadbury, C.; Holden, D.; Aufauvre-Brown, A.; Bainbridge, B.; Cohen, J. Detection of *Aspergillus fumigatus* by polymerase chain reaction. *J. Clin. Microbiol.* **1993**, *31*, 615–621.
(10) Richard, E.; Heutte, N.; Sage, L.; Pottier, D.; Bouchart, V.; Lebailly, P.; Garon, D. Toxigenic fungi and mycotoxins in mature corn silage. *Food Chem. Toxicol.* **2007**, *45*, 2420–2425.
(11) Sutton, P.; Waring, P.; Müllbacher, A. Exacerbation of invasive aspergillosis by the immunosuppressive fungal metabolite, gliotoxin. *Immunol. Cell. Biol.* **1996**, *74*, 318–322.
(12) Pahl, H. L.; Krauss, B.; Schulze-Osthoff, K.; Decker, T.; Traenckner, E. B.; Vogt, M.; Myers, T.; Parks, T.; Warring, P.; Muhlbacher, A.; Czernilofsky, A. P.; Baeuerle, P. A. The immunosuppressive fungal metabolite gliotoxin specifically inhibits transcription factor NF-kappaB. *J. Exp. Med* **1996**, *183*, 1829–1840.
(13) Zhao, Y.; Liu, J.; Wang, J.; Wang, L.; Yin, H.; Tan, R.; Xu, Q. Fumigaclavine C improves concanavalin A-induced liver injury in mice mainly via inhibiting TNF-alpha production and lymphocyte adhesion to extracellular matrices. *J. Pharm. Pharmacol.* **2004**, *56*, 775–782.
(14) Khoufache, K.; Puel, O.; Loiseau, N.; Delaforge, M.; Rivollet, D.; Coste, A.; Cordonnier, C.; Escudier, E.; Botterel, F.; Bretagne, S. Verruculogen associated with *Aspergillus fumigatus* hyphae and conidia modifies the electrophysiological properties of human nasal epithelial cells. *BMC Microbiol.* **2007**, *7*, 5.
(15) Cole, R. J.; Kirksey, J. W.; Dorner, J. W.; Wilson, D. M.; Johnson, J. C.; Johnson, A. N.; Bedell, D. M.; Springer, J. P.; Chexal, K. K.; Clardy, J. C.; Cox, R. H. Mycotoxins produced by *Aspergillus* species isolated from moulded silage. *J. Agric. Food Chem.* **1977**, *25*, 826–830.
(16) Boudra, H.; Morgavi, D. P. Mycotoxin risk evaluation in feeds contaminated by *Aspergillus fumigatus*. *Anim. Feed Sci. Technol.* **2005**, *120*, 113–123.
(17) Lehmann, L.; Wagner, J.; Metzler, M. Estrogenic and clastogenic potential of the mycotoxin alternariol in cultured mammalian cells. *Food Chem. Toxicol.* **2006**, *44*, 398–408.
(18) Gupta, S. K.; Venkitasubramanian, T. A. Production of aflatoxin on soybeans. *Appl. Microbiol.* **1975**, *29*, 834–836.
(19) Pozzi, C. R.; Braghini, R.; Arcaro, J. R.; Zorzete, P.; Israel, A. L.; Pozar, I. O.; Denucci, S.; Correa, B. Mycoflora and occurrence of alternariol and alternariol monomethyl ether in Brazilian sunflower from sowing to harvest. *J. Agric. Food Chem.* **2005**, *53*, 5824–5828.
(20) Schollenberger, M.; Muller, H. M.; Rufle, M.; Suchy, S.; Planck, S.; Drochner, W. Survey of *Fusarium* toxins in foodstuffs of plant origin marketed in Germany. *Int. J. Food Microbiol.* **2005**, *97*, 317–326.
(21) Gamanya, R.; Sibanda, L. Survey of *Fusarium moniliforme* (*F.verticillioides*) and production of fumonisin B_1 in cereal grains and oilseeds in Zimbabwe. *Int. J. Food Microbiol.* **2001**, *71*, 145–149.
(22) Marquardt, R. R.; Frohlich, A. A. A review of recent advances in understanding ochratoxicosis. *J. Anim. Sci.* **1992**, *70*, 3968–3988.
(23) Bayman, P.; Baker, J. L.; Doster, M. A.; Michailides, T. J.; Mahoney, N. E. Ochratoxin production by the *Aspergillus ochraceus* group and *Aspergillus alliaceus*. *Appl. Environ. Microbiol.* **2002**, *68*, 2326–2329.
(24) Adhikari, A.; Reponen, T.; Lee, S. A.; Grinshpun, S. A. Assessment of human exposure to airborne fungi in agricultural confinements: Personal inhalable sampling versus stationary sampling. *Ann. Agric. Environ. Med.* **2004**, *11*, 269–277.
(25) Scott, P. M. Analysis of agricultural commodities and foods for *Alternaria* mycotoxins. *J. AOAC Int.* **2001**, *84*, 1809–1817.
(26) Bush, R. K.; Prochnau, J. J. Alternaria-induced asthma. *J. Allergy Clin. Immunol.* **2004**, *113*, 227–234.
(27) Reboux, G.; Piarroux, R.; Mauny, F.; Madroszyk, A.; Millon, L.; Bardonnet, K.; Dalphin, J. C. Role of molds in farmer's lung disease in Eastern France. *Am. J. Respir. Crit. Care Med.* **2001**, *163*, 1534–1539.
(28) Yoshida, K.; Ando, M.; Ito, K.; Sakata, T.; Arima, K.; Araki, S.; Uchida, K. Hypersensitivity pneumonitis of a mushroom worker due to *Aspergillus glaucus*. *Arch. Environ. Health* **1990**, *45*, 245–247.
(29) Fischer, G.; Müller, T.; Schwalbe, R.; Ostrowski, R.; Dott, W. Species-specific profiles of mycotoxins produced in cultures and associated with conidia of airborne fungi derived from biowaste. *Int. J. Hyg. Environ. Health* **2000**, *203*, 105–116.
(30) Russel, K.; Broadbridge, C.; Murray, S.; Waghorn, D.; Mahoney, A. Gardening can seriously damage your health. *Lancet* **2008**, *371*, 2056.

Received for review October 10, 2008. Revised manuscript received December 10, 2008. Accepted December 23, 2008. This work was supported by a grant from the Ligue Nationale Contre le Cancer (Comité de l'Orne) and the Conseil Général du Calvados. E. Richard and C. Lanier are recipients of a fellowship from the Ministère National de la Recherche et de la Technologie.

JF8031588