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Airborne molds and mycotoxins in *Serpula lacrymans*–damaged homes

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ABSTRACT

For some years, the degradation of homes by the dry rot fungus *Serpula lacrymans* increased. This study described, for the first time, the fungal contamination in homes located in Low–Normandy (France) and damaged by *Serpula lacrymans*. Wood–decaying fungi, airborne molds, fungal species growing on building materials were investigated by cultural and molecular methods. Mycotoxins in the air were quantified by HPLC–MS/MS and the mutagenicity of fungal aerosols was also evaluated using the Ames test. The results showed that *Serpula lacrymans* was detected in the air for one third of homes with sometimes the co–occurrence of other ligninolytic basidiomycetes species like *Donkioporia expansa*. Various molds in the air and on materials (117 and 103 species, respectively) were also identified indicating the complexity of indoor mycoflora. Certain recurrent species like *Aspergillus versicolor* and *Penicillium fellutanum* were observed both on building materials and in the air. The presence of cellulolytic molds in fungal aerosols and on building materials could be used as an indicator of home degradation. Airborne culturable fungal levels were measured up to 5.8×10^5 Colony Forming Units (CFU) per cubic meter of the air (CFU/m^3) depending on the home. Fungal concentrations also depended on the type of collector (filter or liquid) and were significantly correlated with the median of particles between 2–15 µm in size. Two mycotoxins (alternariol and/or ochratoxin A) were observed in 4 homes but no mutagenic activity was found.

Keywords: *Fungal aerosols, indoor environment, molds, mycotoxins, Serpula lacrymans*

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1. Introduction

For some years, the number of houses damaged by wood–rotting fungi such as *Serpula lacrymans* has increased, particularly in Northern Europe and America. *S. lacrymans*, a basidiomycete fungus belonging to Boletales clade, is likely to occur in buildings where there has been persistent water ingress resulting in damp masonry in contact with timber elements (Watkinson and Eastwood, 2012).

Among the species of basidiomyceteous fungi associated with wood decay, dry rot caused by *Serpula lacrymans* is the most serious form of fungal decay in a building and has important consequences on juridical, social and economic levels (Singh, 1994).

Serpula lacrymans fungus shows a mycelium that extensively spreads with water–conducting strands (called rhizomorphs) and forms fruit–bodies as flat as a pancake that produce rusty red spores. The removal of cellulose and hemicelluloses, but not lignin, results in the shrinkage and darkening of the wood into the typical "cubical cracking", and ultimately leaves a powdery brown lignin residue. Wood decomposition by *Serpula lacrymans* involves wood–decomposing enzymes and probably a non–enzymatic attack on cellulose by highly reactive hydroxyl radicals (Shimokawa et al., 2004; Eastwood, 2011).

Species–specific probes and primers have been shown to discriminate between *S. lacrymans* and other wood decay fungi in buildings, using the ITS region of the genome (Schmidt, 2007; Hirosawa et al., 2009). Nevertheless, few of these, concerning molds associated to *Serpula lacrymans*, are known. Although asthma and allergic alveolitis due to basidiospores have been previously described (Herxheimer et al., 1969; O'Brien, 1978; Bryant and Rogers, 1991), no study is currently available on human exposure to fungal aerosols in these damaged homes.

Bioaerosols are particles of biological origin suspended in the air. Airborne molds represent a significant part of the biological contaminants in the air. In an indoor environment, especially where there is dampness and condensation, growing molds exploit the microclimate in ecological niches of buildings and feed on a variety of substrates (Singh, 2001).

Exposure to fungal spores or hyphal fragments has been associated with several types of human or animal health effects including chronic respiratory infections, dry cough, skin and eye irritation, asthmatic symptoms, allergy and various non–specific symptoms (i.e., fever, headache) (Burr, 2001; Douwes et al., 2003; Chapman, 2006). Recent studies in different countries showed that occupants of damp or moldy buildings are at increased risk of respiratory symptoms, respiratory infections and exacerbation of asthma (Fisk et al., 2007; WHO Europe, 2009). However, associations between symptoms and concentrations of fungi seem

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to be dependent on distinctive genera or species (Stark et al., 2005; Rosenbaum et al., 2010). In addition, some of airborne molds can produce mycotoxins in indoor environment. Mycotoxins are able to initiate toxic responses, like mutagenicity, in humans or other vertebrates (Robbins et al., 2000).

These findings emphasize the necessity of a better knowledge on exposure to mold and mycotoxin, particularly in *Serpula lacrymans*–damaged homes. Because some mycotoxins are potentially carcinogenic compounds, the mutagenicity of fungal aerosols collected from damaged buildings should be also evaluated.

The main objective of the current study was to describe fungal contamination in *Serpula lacrymans*–damaged homes in order to assess the occupant's exposure. The three specific aims were (1) to describe the fungal profile of bioaerosols and building materials collected in *Serpula lacrymans*–damaged homes, (2) to assess human exposure to indoors mycotoxins, and (3) to evaluate the mutagenicity of fungal aerosols.

2. Materials and Methods

2.1. Selection of homes and fungal aerosols sampling methods

The study was based on the investigation of 20 homes damaged by wood–rotting fungi. These homes were selected by an expert engineer on buildings and located in Low–Normandy (France). The major types of contaminated buildings were homes with elevated moisture content, insufficient ventilation, and improper renovation. Episodes of water infiltration or water damage were the main causes of *Serpula lacrymans* outbreaks (Table 1).

Two rooms, one with visible damage caused by *Serpula lacrymans* (room 1), and the other one without apparent damage (room 2) were evaluated. In each home, fungal aerosols were collected in the middle of room at 1 meter height. Aerosols were then studied for their physical characteristics (particle number concentration in 15 channels from 0.3 µm to 20 µm, relative humidity <95% and temperature from 0 to +40 °C) and analyzed for their contents in wood–decaying fungi, molds and mycotoxins. Information on the homes' characteristics and history, and health symptoms questionnaires were also collected when the home was still occupied (Table 1). During the visits, particles between 2 and 15 µm, relative humidity (%) and temperature (°C) were monitored every 6 s during 3 h by a portable aerosol spectrometer (Model 1.108, Grimm Technologies, Inc., Douglasville, GA, USA) (Table 2).

Fungal aerosols were collected using two different methods:

(1) A sterile PTFE filter of 0.2 µm pore size (Sartorius Biolab, Palaiseau, France) mounted in 47 mm diameter filter holders (Pall Corporation, Ann Arbor, MI, USA) and connected to personal air pumps (SKC 224 52TXK, Arelco, France) calibrated to draw 2 L/min for 3 h. These three filters were used per room: the first for mold quantification, the second for mycotoxin quantification and the third for mutagenicity evaluation.

Table 1. *Description of homes investigated*

Home	Brief Description	Related Symptoms
1	Residential building built in 1920 Water damage	Unoccupied
2	Renovated residential building Water infiltrations	Unoccupied
3	Wooden house built in 2007 Water infiltrations	No
4	Stone house built in 1850 Water infiltrations	Unoccupied
5	Castle built in the 18th century	No
6	House built in 1964	No
7	Farm building renovated in living house Water infiltrations	No
8	Renovated 18th century house Water infiltrations	Sore throat, itching, urticaria
9	Farm building renovated in living house Water infiltrations	Unoccupied
10	Residential building built in 1950 Water damage	Unoccupied
11	House built in 1850 Water infiltrations	Unoccupied
12	Renovated 19th century house Water infiltrations	Cough, breathing difficulties
13	Residential building built in 1920 Water damage	Blocked nose, sore throat, cough, headache, muscular pain, asthma, breathlessness, urticaria, sleeping disorders, fatigue
14	House built in 1680 Water infiltrations	No
15	House built in 1960 Water infiltrations	Eye irritation, sore throat, headache, cough, sinusitis, breathlessness, sleeping disorders
16	House built in 1902 Water infiltrations	Eye irritation , sore throat, cough, headache, muscular pain, flu–like syndrome, sinusitis, asthma, breathing difficulties, itching, eczema, sleeping disorders
17	House built in 1900 Water infiltrations	Blocked nose, sore throat, cough, headache, sinusitis, breathlessness, urticaria
18	Renovated 19th century house	Blocked nose, conjunctivitis, sore throat, headache, asthma, breathing difficulties, itching , sleeping disorders, sneezing
19	Residential building built in 1850 Air openings blocked	Unoccupied
20	House built in 1955 Water infiltrations	No

Table 2. *Physical characteristics of homes investigated in this study*

Home	Room [a]	Median [b] Particle Counts Between 2 and 15 µm/L air	Median [b] Temperature (°C)	Median [b] Relative Humidity (%)
01	1	5 354	10.8	42.4
	2	3 623	9.0	44.2
02	1	888	9.5	65.2
	2	1 503	9.8	62.9
03	1	232	19.8	50.8
	2	235	17.1	57.9
04	1	1 598	16.7	58.1
	2	644	15.6	62.3
05	1	143	21.4	42.8
	2	71	19.3	51.6
06	1	3 709	22.2	50.0
	2	634	21.9	45.4
07	1	1 653	21.3	56.1
	2	564	20.1	57.0
08	1	409	20.1	61.2
	2	490	20.0	59.6
09	1	6 326	21.4	60.5
	2	4 106	21.0	55.3
10	1	2 010	20.5	66.8
	2	807	18.2	30.4
11	1	1 015	10.1	68.3
	2	1 588	12.1	58.0
12	1	974	11.6	62.6
	2	1 766	10.8	66.3
13	1	660	20.0	43.1
	2	599	19.9	42.9
14	1	716	23.1	56.1
	2	713	23.0	55.0
15	1	635	21.7	62.7
	2	756	26.0	38.4
16	1	1 124	23.8	50.5
	2	649	22.8	51.7
17	1	1 723	24.3	58.9
	2	248	23.1	55.4
18	1	486	20.2	62.3
	2	391	19.4	60.7
19	1	689	16.0	76.3
	2	774	15.1	74.7
20	1	433	20.8	49.9
	2	533	21.0	46.5

[a] *Room 1: Visible Serpula lacrymans damage; room 2: non–visible Serpula lacrymans damage*
[b] *Median of the measurements (every 6 s during 3 h)*

(2) A sterile liquid with a cyclonic air sampler Coriolis µ® (Bertin Technologies, France) calibrated to draw air at 300 L/min for 10 min. Two liquid samples were collected: the first for wood–decaying basidiomycetes detection and the second for mold quantification.

2.2. Analysis of fungal aerosols

Detection of wood–decaying basidiomycetes. Because basidiomycetes do not easily grow in culture media, a molecular method was used for their detection. DNA was extracted using a Nucleospin Plant (Macherey Nagel, Duren, Germany). First, the liquid sample (15 mL) collected by Coriolis µ® sampler was centrifuged at 14 000 rpm, for 5 min. The pellet was then placed into Qiagen TissueLyser (Valencia, CA, USA) with glass beads and 250 µL of lysis buffer for 15 min at 30 Hz. Then, 400 µL of chloroform (Sigma–Aldrich, St. Louis, MO, USA) was added with 20 µL of proteinase K at 10 mg/mL (Sigma–Aldrich, St. Louis, MO, USA). The aqueous phase was then recovered by centrifugation (5 min at 14 000 rpm), incubated for 30 min at 60 °C and filtered. The DNA was precipitated using 600 µL of buffer and 400 µL of absolute ethanol and washed using various washing buffers supplied by the manufacturer. An elution was finally performed in two phases with 2x50 µL of elution buffer pre–incubated at 70 °C.

The universal primer ITS–1 (forward) combined to each taxon primers (reverse) was used to differentiate of the four major wood–rot fungi namely: *Coniophora puteana*, *Donkioporia expansa*, *Serpula himantioides*, and *S. lacrymans* (Moreth and Schmidt, 2000; Schmidt and Moreth, 2000).

The reaction mixture consisted of 1xPCR (polymerase chain reaction) buffer supplemented with 1.5 mM $MgCl_2$ (Roche for Applied Biosystems, New Jersey, USA) and 0.1% Triton X–100 (Sigma–Aldrich, St. Louis, MO, USA), 0.2 mM deoxynucleoside triphosphates (Applied Biosystems, Warington, UK), 0.5 µM each forward and reverse primers (Eurogentec, Seraing, Belgique) and 2.5 U *Taq* polymerase (Roche for Applied Biosystems, New Jersey, USA). The sensitivity was 100 pg for *Serpula lacrymans*, 10 pg for *Serpula himantioides* and *Donkioporia expansa*, and 1 pg for *Coniophora puteana*. Each experiment included a positive control of a known amount of template DNA (standard strain) and a negative control without DNA.

Each reaction mixture was heated to 94 °C for 7 min. A total of 35 amplifications were carried out on a programmable DNA thermal cycler (Mastercycler gradient Eppendorf, NY, USA). To denature the genomic DNA, reactions were held for 1 min at 94 °C followed by 1 min at 55 °C for annealing and 1 min at 72 °C for extension. Before storage at 4 °C, a final extension of 7 min at 72 °C was performed.

The PCR products were analyzed by electrophoresis on 1% agarose gel in 1xTBE (Sigma–Aldrich, St. Louis, MO, USA) stained with 0.30 µg/mL of ethidium bromide.

Identification and quantification of airborne molds. PTFE filters were suspended in 5 mL of sterile water containing Tween 80 (0.05%, w/v) (Sigma–Aldrich, St. Louis, MO, USA) and shaked for 30 min at 420 rpm. The liquid sample collected from Coriolis µ® was directly used. Both microbial suspensions were diluted up to 10^{-3}. One mL aliquots were deposited into triplicate plates, and MEA [malt extract (1.5%) / agar (1.5%)] supplemented with chlor-amphenicol (0.05%, w/v) was poured. The plates were incubated at 25 °C and 30 °C for 7 days, and checked daily. The resulting colonies were counted and reported as Colony Forming Units (CFU)/m^3. Each fungal colony was isolated, and purified on MEA medium. Identification of all fungal isolates was done using macroscopic and microscopic features following the previous studies (Booth, 1966; Pitt, 1979; Domsch et al., 1980; Von Arx, 1981; Pitt, 2000; Klich, 2002; Samson et al., 2002; Samson and Frisvad, 2004). All plates were also observed during 14 days in order to determine the possible presence of *Stachybotrys chartarum*.

Identification of molds associated building materials. Building materials (*n*=122 and 29) with visible mold growth were sampled (from rooms 1 and 2, respectively) using swabs. The identification of fungal species was done as previously described after 7 days of incubation at different temperatures (25, 30 and 37 °C) on different media: MEA, PDA and VM (MEA complemented with 0.01 g/L of malachite green).

Multi–mycotoxin analysis of fungal aerosols. Twenty mycotoxins were searched from the fungal aerosols. The mycotoxins were extracted from the PTFE filters with 10 mL of methanol/water/ formic acid (80/20/0.1). The solutions were kept in an ultrasonic bath for 3 min, and then shaken in a multi–tube vortexer. A second extraction was realized with 10 mL of dichloromethane/ethyl acetate (50/50). The two extracts were pooled and evaporated to dryness with a Buchi evaporator and then under nitrogen. The final residue was dissolved in 0.5 mL of a mixture of acetonitrile/water (10/90) and then filtered through Millex HV 0.45 µm before injection into HPLC–MS/MS.

Mycotoxins were quantified by high performance liquid chromatography coupled to mass spectrometry (HPLC–MS/MS) in multiple reaction monitoring (MRM) mode. Liquid chromatography was performed using an Agilent Technologies 1200 HPLC system coupled to a triple quadrupole mass spectrometer (6460 series, Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray interface, operated in the positive and negative modes. The MassHunter B.02.00 software was used for data processing. The analytes were analyzed using two chromatographic methods.

Eleven mycotoxins (aflatoxins B_1, B_2, G_1, G_2, M_1, diacetoxy-scirpenol, gliotoxin, mycophenolic acid, neosolaniol, ochratoxin A, T–2 toxin) were separated (Method 1) onto Zorbax SB, Rapid Reso-lution HT–C_{18} column (1.7 µm, 50x2 mm; Agilent Technologies) at 60 °C. Fumonisin B_1–$^{13}C_{34}$ was used as the internal standard. The injection volume of the samples on the analytical column was 10 µL. The mobile phase consisted of a variable mixture of acetonitrile (solvent A) and water added with formic acid 1% (solvent B) at a flow rate of 0.4 ml/min. A linear gradient was run starting with 10% to 100% solvent A over 10 min and staying at 100% over 1 min.

The mass spectrometer was operated in positive mode using dynamic multiple reaction monitoring (MRM). The nebulizer and desolvatation gases were nitrogen heated at 300 °C at 10 L/min and 400 °C at 12 L/min, respectively.

Nine mycotoxins (alternariol, deoxynivalenol, deepoxydeoxy-nivalenol, 3–acetyldeoxynivalenol, 15–acetyldeoxynivalenol, fusa-renon X, HT–2 toxin, verrucarol, zearalenone) were separated (Method 2) onto a Zorbax Eclipse Plus, Rapid Resolution HD–C_{18} column (1.7 µm, 50x2 mm; Agilent Technologies) at 60 °C. Deoxynivalenol–$^{13}C_{15}$ was used as the internal standard. The injection volume of the samples on the analytical column was 20 µL. The mobile phase consisted of a variable mixture of methanol (solvent A) and water (solvent B) at a flow rate of 0.4 mL/min. A linear gradient was run, starting with 10% to 100% solvent A over 10 min and staying at 100% over 1 min.

The mass spectrometer was operated in both negative and positive modes using multiple reaction monitoring (MRM). Three retention windows were defined according to the retention time and the optimized ESI mode. The nebulizer gas and desolvatation gases were nitrogen heated at 250 °C at 10 L/min (except for the third retention window, at 12 L/min) and 400 °C at 12 L/min, respectively.

Other common parameters used for the mass spectrometer were as follow: capillary voltage, 4.0 kV; pressure of nebulization, 45 psi; nozzle voltage, 300 V.

The most abundant product ion after collision–induced fragmentation was used for quantitative purposes, and the second product ion for confirmation. The linearity was done by spiking increasing concentrations (triplicate) of the mycotoxin standards (0.1 to 50 µg/L). The quantification and detection limits (QL and DL) were determined by spiked samples based on signal to noise ratio of 10:1 for quantification, and 3:1 for detection limit. Recoveries and quantification limits were presented in Table 3.

Mutagenicity of fungal aerosols using the Ames test. The PTFE filters were suspended twice in 30 mL methanol acidified with acetic acid (0.5%). The solutions were kept in an ultrasonic bath for 3 min, and then shaken for 10 min in a multi–tube vortexer. The supernatant was transferred to an appropriate glass tube. All extracts were evaporated in a parallel evaporator (Syncore polyvap, Buchi Labotechnik G, Flawil, Switzerland) and then dried under a stream of nitrogen. The final residue was dissolved in 550 µL DMSO and then filtered through Millex HV 0.45 µm. Each filter extract was tested in triplicate.

***Table 3.** Recoveries and quantification limits of mycotoxins in fungal aerosols*

Mycotoxin [a]	Recovery (%)	Quantification limit (ng/filter)
15AcDON	100.70	0.15
3AcDON	97.90	0.15
AFB1	79.20	0.15
AFB2	93.40	0.15
AFG1	77.10	0.15
AFG2	79.40	0.15
AFM1	85.20	0.15
ALT	64.90	0.15
DAS	90.90	0.05
DOM–1	90.60	0.15
DON	67.60	0.15
FUS–X	84.90	0.50
GLIO	75.20	0.50
HT2	100.70	0.15
MPA	80.20	0.15
NEO	101.10	0.05
OTA	76.90	0.15
T2	83.00	0.05
VERC	87.50	0.50
ZEA	56.00	0.15

[a] *AFB1 – aflatoxin B_1, AFB2 – aflatoxin B_2, AFG1 – aflatoxin G_1, AFG2 – aflatoxin G_2, AFM1 – aflatoxin M_1, ALT – alternariol, DAS – diacetoxyscirpenol, DOM1 – deepoxydeoxynivalenol, DON – deoxynivalenol, 3AcDON – 3acetyldeoxynivalenol, 15AcDON – 15acetyldeoxynivalenol, FUS–X – fusarenon X, GLIO – gliotoxin, HT2 – HT–2 toxin, MPA – mycophenolic acid, NEO – neosolaniol, OTA – ochratoxin A, T2 – T–2 toxin, VERC – verrucarol, ZEA – zearalenone.*

The preincubation method of the *Salmonella typhimurium* assay was used according to Mortelmans and Zeiger (2000). The strains were grown in Nutrient Broth No. 2 (Oxoid ltd., Basingstoke, England) with a 12 h shaking at 37 °C. Ten µL of the bioaerosol extract were mixed with 100 µL of the bacterial culture and 100 µL of 5% S9 mix (liver homogenate for metabolic activation) when appropriate. The S9 fraction was obtained from Aroclor 1254–induced male Sprague–Dawley rats, and supplied by Moltox (Boone, NC, USA). After 60 min of agitation (185 rpm) at 37 °C, 2 mL of molten agar with 10% histidine and biotin was added to the tubes and poured on minimal glucose plates. After 48 h of incubation at 37 °C, the number of revertants was counted using the Noesis software (Saint Aubin, France). Because mutagens can damage DNA through various mechanisms, 3 strains were tested, enabling the detection of different types of mutations: TA98 (frameshift damages), TA100 (base–pair substitutions) and TA102 (base–pair substitution mainly consecutive to oxidative damage).

Statistical procedures. All statistical analyses were made with the SAS system version 9.3 (SAS Institute Inc., Cary, NC, USA). The hypotheses on quantitative variables were tested by GLM (general linear models) procedure. When non parametric tests were required, the Spearman rank correlation (corr procedure) was used to determine the relationships between CFU, and particles count, temperature, relative humidity. In addition, Wilcoxon test (NPar1Way procedure) was used to determine the effect of experimental conditions on airborne molds contamination.

3. Results

3.1. Wood–damaging fungi in fungal aerosols

Nine houses contained wood–rotting basidiomycete fungi in the indoor air. Air bio–contamination was mainly due to *Serpula lacrymans* with sometimes the co–occurrence of other ligninolytic strains like *Donkioporia expansa* (*n*=4 homes) *or Serpula himantioides* (*n*=1 home). *Coniophora puteana* was not detected in bioaerosols.

Serpula lacrymans was detected in both non–damaged and damaged rooms of 6 homes which suggest a dissemination of the spores inside the homes. The detection of *Serpula lacrymans* in the air is linked to the presence of sporulating fruiting body i.e. active sporophores (homes 1, 2, 4 and 7), and proliferating mycelium (homes 12, 15, 19). Active sporophore (home 4) and proliferating mycelium (home 19) of *Serpula lacrymans* are shown in Figures 1a and 1b, respectively.

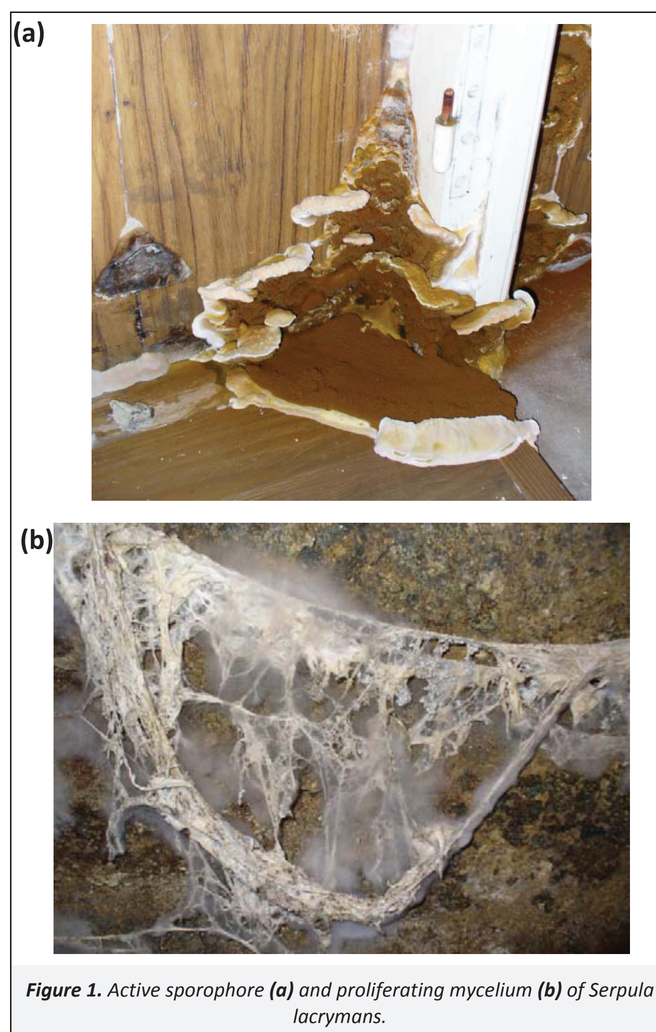

***Figure 1.** Active sporophore **(a)** and proliferating mycelium **(b)** of Serpula lacrymans.*

3.2. Molds in fungal aerosols

Fungal biodiversity. In rooms with or without visible damage of *Serpula lacrymans*, 86 and 89 mold species were identified, respectively. The mycoflora was in particular constituted by 38 *Penicillia* and related genera (*Eupenicillium* and *Talaromyces*), 13 *Aspergilli* and related genera (*Emericella*, *Eurotium*). The number of species per home varied from 15 to 37. Some of them were recurrent and similar in the 2 rooms: *Aspergillus fumigatus*, *A. versicolor* and *Penicillium fellutanum* (Table 4).

Among all species, allergenic genera (*Alternaria*, *Cladosporium*, *Ulocladium*) were observed. Some strains belonging to *Alternaria alternata* and *Aspergillus melleus* were able to produce *in vitro* alternariol (mean concentration: 16 µg/g medium) and ochratoxin A (mean concentration: 197 ng/g medium), respectively.

Table 4. *Recurrent molds in fungal aerosols collected from Serpula lacrymans–damaged homes*

Fungal species	Relative frequency (%) in Room 1 [a]	Relative frequency (%) in Room 2 [a]
Alternaria alternata	30	30
Aspergillus fumigatus	55	85
Aspergillus versicolor	90	75
Chaetomium globosum	20	30
Cladosporium cladosporioides	50	50
Cladosporium herbarum	30	30
Epicoccum purpurascens	35	20
Penicillium brevicompactum	45	40
Penicillium chrysogenum	45	60
Penicillium expansum	35	45
Penicillium fellutanum	95	80
Penicillum piceum	35	35
Trichoderma viride	55	30
Ulocladium chartarum	30	25

[a] *Room 1: visible Serpula lacrymans damage; room 2: non–visible Serpula lacrymans damage*

Species known as cellulolytic molds were observed: *Epicoccum purpurascens* and *Trichoderma viride* were especially identified in the room with visible *Serpula lacrymans* damage, and *Chaetomium globosum* was identified in both rooms.

Quantification of culturable molds. The concentrations of airborne molds (total CFU) are presented in Table 5. The mean concentrations of culturable airborne fungi did not significantly differed between the damaged and non–damaged rooms (P=0.1214).

The levels of total culturable molds varied from $0.5x10^1$ to $5.8x10^5$ CFU per cubic meter of air, according to the home. Home 1 showed the highest level of molds between $6.4x10^4$ and $5.8x10^5$ CFU/m^3. In a previous study (unpublished data), we performed the analysis of homes without *Serpula lacrymans*. Results indicated that levels of total culturable molds never exceed 9.5×10^1 CFU/m^3. In these homes, we always noted the absence of cellulolytic species like *Trichoderma viride* and *Stachybotrys chartarum*.

A significant difference was observed between the 2 types of sampling (P<0.0001). The sampling of fungal aerosols in sterile liquid was more successful. The studies of correlation showed that the total CFU was significantly correlated to the median of particles between 2 to 15 µm in size (P=0.0461, r=0.32) and indicated a negative correlation between total CFU and temperature (P=0.01, r=–0.40). In contrast, no correlation was found with the relative humidity (P=0.179).

Table 6 exposes airborne fungal concentrations of some species previously described as recurrent molds in fungal aerosols. While recurrent molds like *Aspergillus versicolor* and *Penicillium fellutanum* were also predominant from a quantitative point of view, the concentration of other species like *Aspergillus fumigatus* appeared to be weak. On the contrary, some species like *Aureobasidium pullulans* or *Exophiala moniliae* were not considered as recurrent strains, but could sometimes represent high concentrations in some homes (up to $1.4x10^4$ and $9.4x10^3$ CFU/m^3, respectively).

Our experimental conditions were tested during this study: air sampling method (solid i.e. filter *versus* liquid) and fungal growth temperature (25 and 30 °C). Table 7 presents the effects of these parameters on the levels of 8 fungal species selected for their potential toxicity. As previously described for the total CFU, the statistical analysis showed that the sampling of fungal aerosols in liquid allowed the recovery of more culturable molds (*Cladosporium cladosporioides* and *Aureobasidium pullulans*). The concentrations of these 2 species were weak after sampling on filter. In contrast, the concentration of *Aspergillus fumigatus* was higher after sampling on filter. A culture at 25 °C significantly quantified more CFU of *Aspergillus versicolor*, *Cladosporium cladosporioides* and *Cladosporium herbarum* than a culture at 30 °C.

Table 5. *Mold spore concentration (total CFU/m^3) in fungal aerosols of homes according to room and sampling method (filter or liquid)*

Home	Room [a]	PTFE filter Total CFU/m^3	Sterile liquid Total CFU/m^3
1	1	$6.4x10^4$	$5.8x10^5$
	2	$1.2x10^5$	$4.7x10^5$
2	1	$4.6x10^3$	$7.8x10^3$
	2	$2.6x10^3$	$8.9x10^3$
3	1	$1.1x10^3$	$2.8x10^4$
	2	$6.7x10^2$	$2.9x10^4$
4	1	$5.5x10^3$	$6.8x10^2$
	2	$1.5x10^3$	$6.1x10^2$
5	1	$2.1x10^1$	$6.7x10^1$
	2	$3.0x10^1$	$1.7x10^1$
6	1	$8.8x10^1$	$2.1x10^3$
	2	$6.8x10^1$	$4.4x10^2$
7	1	$4.0x10^4$	$1.3x10^3$
	2	$6.5x10^3$	$3.6x10^2$
8	1	$5.2x10^3$	$2.5x10^3$
	2	$1.9x10^2$	$2.5x10^3$
9	1	$1.0x10^2$	$6.8x10^2$
	2	$2.9x10^2$	$8.2x10^2$
10	1	$1.9x10^3$	$5.2x10^2$
	2	$1.2x10^3$	$1.3x10^4$
11	1	$4.6x10^1$	$2.6x10^1$
	2	$4.6x10^1$	$3.3x10^1$
12	1	$5.7x10^3$	$2.4x10^3$
	2	$1.6x10^2$	$3.0x10^3$
13	1	$1.9x10^1$	$1.5x10^1$
	2	$0.5x10^1$	$1.6x10^1$
14	1	$5.3x10^1$	$1.9x10^3$
	2	$0.5x10^1$	$4.8x10^1$
15	1	$2.6x10^2$	$2.7x10^3$
	2	$1.1x10^2$	$1.0x10^2$
16	1	$2.6x10^2$	$2.0x10^2$
	2	$5.8x10^2$	$2.1x10^2$
17	1	$1.0x10^2$	$5.6x10^2$
	2	$3.0x10^1$	$4.4x10^2$
18	1	$0.9x10^1$	$4.6x10^1$
	2	$1.9x10^1$	$3.3x10^1$
19	1	$5.1x10^2$	$9.3x10^3$
	2	$6.7x10^2$	$1.1x10^3$
20	1	$1.6x10^1$	$2.4x10^1$
	2	$1.2x10^1$	$2.6x10^1$
Total homes (mean)	1	$6.5x10^3$	$3.1x10^4$
Total homes (mean)	2	$6.5x10^3$	$2.6x10^4$

[a] *Room 1: visible Serpula lacrymans damage; room 2: non–visible Serpula lacrymans damage*

Table 6. *Concentration values (CFU/m^3) of recurrent species collected in fungal aerosols of homes*

Fungal species	Room [a]	Minimum CFU/m^3	Maximum CFU/m^3	Mean CFU/m^3
Aspergillus fumigatus	1	nd	2.3x10^2	3.4x10^1
	2	0.1x10^1	3.2x10^2	3.1x10^1
Aspergillus versicolor	1	0.1x10^1	1.2x10^5	7.6x10^3
	2	0.1x10^1	1.1x10^5	7.6x10^3
Cladosporium cladosporioides	1	0.1x10^1	1.1x10^2	2.9x10^1
	2	0.1x10^1	1.7x10^2	6.7x10^1
Epicoccum purpurascens	1	0.1x10^1	5.0x10^1	1.9x10^1
	2	0.3x10^1	2.8x10^1	1.6x10^1
Penicillium chrysogenum	1	0.3x10^1	5.6x10^2	1.2x10^2
	2	nd	1.2x10^2	2.4x10^1
Penicillium fellutanum	1	nd	1.9x10^5	1.2x10^4
	2	0.1x10^1	1.7x10^5	1.1x10^4
Trichoderma viride	1	nd	1.1x10^2	3.0x10^1
	2	nd	2.8x10^2	5.8x10^1

[a] Room 1: visible Serpula lacrymans damage; room 2: non–visible Serpula lacrymans damage
nd: not detected

Table 7*. Effects of experimental conditions on airbone mold concentrations (CFU/m^3)*

Fungal species	Sampling mode			Growth temperature		
	Solid Mean CFU/m^3	Liquid Mean CFU/m^3	P	25 °C Mean CFU/m^3	30 °C Mean CFU/m^3	P
Aspergillus fumigatus	4.2x10^1	2.2x10^1	0.0101 [a]	3.1x10^1	3.3x10^1	0.9265
Aspergillus melleus	1.8x10^2	6.7x10^1	0.9185	4.7x10^2	2.8x10^2	0.7874
Aspergillus versicolor	4.4x10^3	1.1x10^4	0.2465	1.1x10^4	4.4x10^3	0.0236 [a]
Aureobasidium pullulans	0.08x10^1	7.5x10^3	0.0001 [a]	6.0x10^3	1.5x10^3	0.8060
Cladosporium cladosporioides	0.06x10^1	1.0x10^2	0.0256 [a]	1.0x10^2	0.05x10^1	0.0020 [a]
Cladosporium herbarum	0.6x10^1	3.4x10^2	0.3716	3.5x10^2	0.1x10^1	0.0011 [a]
Stachybotrys chartarum	1.1x10^2	2.6x10^1	0.2470	2.9x10^1	1.1x10^2	0.0823
Trichoderma viride	5.4x10^1	2.7x10^1	0.8777	4.5x10^1	3.6x10^1	0.2797

[a] Statistically significant difference (Wilcoxon test)

3.3. Molds on building materials

As a whole, 151 building materials were analyzed which allowed the determination of 103 fungal species. Molds were mainly associated with wood (45%), mortar (18%), plastic (7%), and wallpaper (5%) samples.

Table 8 presents the most frequent molds found on building materials. *Aspergillus versicolor*, *Cladosporium cladosporioides*, *Penicillium chrysogenum* and *P. fellutanum* were recurrent on building materials located in damaged and non–damaged rooms and have also been identified in fungal aerosols. The frequent occurrence of cellulolytic species *Trichoderma viride* in damaged rooms suggests its probable role in home degradation in cooperation with wood–rotting fungi.

3.4. Mycotoxins in fungal aerosols and mutagenicity

Two mycotoxins, alternariol and ochratoxin A, were found in 4 homes (Table 9). In all homes, the results of mutagenicity were negative for the tests with strains TA 98, TA 100 and TA 102.

4. Discussion

4.1. Fungal diversity

This study constitutes a detailed description of mycoflora (117 species were identified) in *Serpula lacrymans*–damaged homes. *Aspergillus versicolor*, *Cladosporium cladosporioides*, *Penicillium chrysogenum*, *P. fellutanum* and *Trichoderma viride* were the most frequently identified strains in both building materials and fungal aerosols. Similarly, *Penicillium chrysogenum* and *Aspergillus versicolor* are known as common fungal species in water–damaged buildings (Andersen et al., 2011).

In our study, *Stachybotrys* was detected in 15% of *Serpula lacrymans*–damaged rooms. The three homes with *Stachybotrys chartarum* were characterized by high level of humidity (maximum values of relative humidity: 65.9, 66.7 and 77%). The sampling methods (filter or liquid) showed no significant effect on *Stachybotrys* quantification. *Stachybotrys chartarum* is rarely isolated from air because this mold forms sticky masses of spores which are not easily aerosolized and because of its slow–growth on culture media (Kuhn et al., 2005). In comparison with a previous study on water–damaged buildings, Bloom et al. (2009) have identified *Stachybotrys* in almost all houses using PCR (polymerase chain reaction). These results would seem to indicate the better effectiveness of PCR for *Stachybotrys* detection.

These results could be used to focus on future research on the toxigenicity and ecology of recurrent strains. For example, a species like *Trichoderma viride*, widely known as a cellulases producer (Beldman et al., 1985), was more frequently detected in *Serpula lacrymans*–damaged rooms indicating its possible role in home degradation, in cooperation with wood–rotting fungi. The genus *Trichoderma* could be consequently considered as a fungal indicator of home degradation.

***Table 8**. Relative frequency of molds on building materials in Serpula lacrymans–damaged homes*

Fungal species [a]	Relative frequency (%) in Room 1 [b]	Relative frequency (%) in Room 2 [b]
Aspergillus fumigatus	10.7	10.3
Aspergillus melleus	15.6	3.4
Aspergillus versicolor	25.4	24.1
Chaetomium globosum	5.7	17.2
Cladosporium cladosporioides	24.6	34.5
Cladosporium herbarum	18.9	6.9
Penicillium brevicompactum	8.2	17.2
Penicillium chrysogenum	27.9	31.0
Penicillium corylophilum	3.3	10.3
Penicillium fellutanum	23.0	17.2
Rhizopus stolonifer	3.3	13.8
Trichoderma viride	36.1	17.2

[a] *Species identified at least on 10% of building materials in rooms 1 or 2*
[b] *122 building materials from room 1 (visible Serpula lacrymans damage); 29 building materials from room 2 (non–visible Serpula lacrymans damage)*

***Table 9.** Mycotoxins found in Serpula lacrymans–damaged homes*

Home	Room [a]	Mycotoxin(s)	Concentration(s) (ng/filter)
10	1	alternariol	<QL [b]
	2	alternariol	<QL
11	1	alternariol; ochratoxin A	0.30; 0.34
	2	alternariol; ochratoxin A	0.18; 0.15
12	1	alternariol	0.18
	2	alternariol	0.16
13	1	alternariol	<QL
	2	alternariol	0.15

[a] *Room 1: visible Serpula lacrymans damage; room 2: non–visible Serpula lacrymans damage*
[b] *QL : Quantification limit (0.15 ng/filter)*

The circulation of fungal spores between damaged and non–damaged rooms inside homes was supported by the data obtained after sampling airborne wood–damaging basidiomycetes and associated molds. Moreover, *Serpula lacrymans* (dry rot fungus) was sometimes associated with others wood–damaging fungi like *Donkioporia expansa* (white rot fungus) and *Chaetomium globosum* (soft rot fungus). The white–rot fungus *Donkioporia expansa*, known as a lignin–decaying species, is characterized by a more discreet development, and therefore it is not easily observed by a simple visual examination. The use of a molecular method allowed its detection in fungal aerosols and showed that the contamination by wood–damaging fungi and thus the occupants' exposure can involve several species of basidiomycetes during home degradation.

4.2. Quantification of airborne molds

The mold concentrations (CFU) in *Serpula lacrymans*–damaged homes were strongly home–dependent. The home's characteristics, the background of previous damages and renovations, the stages of development of *Serpula lacrymans* (sporulation, non–growing or actively growing mycelium) are essential parts for a better understanding of each home contamination. Concerning homes 2 to 20, the mean mold concentration was 3.2×10^3 CFU/m^3 which was greater than the mean value (1.1×10^3 CFU/m^3) obtained after impaction in dwellings with visible molds and symptoms in occupants (Reboux et al., 2009).

In home 1, the highest levels of molds were quantified from 6.4×10^4 to 5.8×10^5 CFU/m^3 according to the type of air collector (filter or liquid). These concentrations were especially comparable to the mold concentration found in fungal aerosols collected in homes after flooding (1.1×10^4 to 6.5×10^5 CFU/m^3) (Solomon et al., 2006). Based on works of Eduard (2009), a level of 10^5 spores/m^3 could be considered as consistent LOELs (Lowest Observed Effect Levels) for diverse fungal species in non–sensitized populations.

4.3. Airborne mycotoxins

Two airborne mycotoxins, alternariol and ochratoxin A, were found in this study. This result was supported by the mycotoxigenic assessment that demonstrated the ability of airborne molds isolates belonging to *Alternaria alternata* and *Aspergillus melleus* to produce alternariol and ochratoxin A *in vitro*, respectively. These results highlighted the potential health hazards posed by toxigenic airborne molds in *Serpula lacrymans*–damaged homes. Nevertheless, the low levels of mycotoxins in fungal aerosols could explain the absence of mutagenicity in detected fungal aerosols.

Verrucarol and sterigmatocystin have been identified in a few samples of dust collected from water–damaged homes in New Orleans (Bloom et al., 2009). Several fungal metabolites were also detected on building materials (Taubel et al., 2011). Among these mycotoxins, alternariol, ochratoxin A, mycophenolic acid and sterigmatocystin have been reported. Recently, ochratoxin A has been also detected in the environmental and biological samples of occupants in a water–damage home (Thrasher et al., 2012). Our study showed that multi–analyte tandem mass spectrometry–based methodology was useful to search for mycotoxins in fungal aerosols, and so allowed to assess the occupants' exposure to indoor mycotoxins. Nevertheless, future studies are needed to better understand the mycotoxins levels in indoor aerosols during long–term sampling.

4.4. Technical implications

The presence of cellulolytic molds in fungal aerosols and on building materials could be used as an indicator of home degradation. The detection of several wood–rotting basidiomycetes in homes showed that both microscopic and molecular (PCR) analyses should be performed in order to precisely evaluate fungal contamination. In addition, this study also suggests that the amount of particles between 2 to 15 µm in size could be a useful tool to assess airborne mold contamination in *Serpula lacrymans*–damaged homes.

In comparison to impaction, sampling on filter or in liquid allows flexibility in dealing with unpredictable levels of spores by permitting serial dilution of the wash solution. Impaction becomes overloaded in conditions of high airborne concentrations (Stetzenbach and Buttner, 2005). As described by Jensen et al. (1994), filtration techniques are used for the collection of certain fungi and endospore–forming bacteria that are desiccation–resistant. In our study, for an optimal exposure assessment, the use of a sterile liquid–based sampling technique of fungal aerosols is recommended because of a better recovery of molds from fungal aerosols. This efficiency was particularly observed with some species like *Cladosporium cladosporioides* or *Aureobasidium pullulans*. Although the quantification of *Aspergillus fumigatus* from the filter was better, a monitoring using a cyclonic–based liquid air collector followed by cultivation at 25 °C would be preferable to assess global fungal exposure in *Serpula lacrymans*–damaged buildings.

5. Conclusions

Serpula lacrymans–damaged homes showed a diversity of molds, sometimes at high levels and even in apparently non–damaged rooms. The detection of cellulolytic species in fungal aerosols and on buildings materials could be useful indicators of home degradation. However, more studies are required to confirm the fungal species which could be used for *Serpula lacrymans*–damaged homes management.

The diversity and complexity of this "fungal indoor ecosystem" including wood–damaging fungi, associated–molds and mycotoxins, could highlight a potential health hazard and need to define guidelines for evaluation and remediation of *Serpula lacrymans*–damaged buildings.

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