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## Relative efficiencies of two air sampling methods and three culture conditions for the assessment of airborne culturable fungi in a poultry farmhouse in France

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### ABSTRACT

Fungal elements represent a significant part of the biological contaminants that could be detected in the air of animal facilities. The aim of this study was to assess the relative efficiencies of two air sampling methods and three culture conditions for the quantification of airborne culturable fungi in a poultry farmhouse in France. Air samples were collected every week throughout a 15-week period. Two devices were simultaneously used—a rotative cup air sampler (CIP 10-M, Arelco, France) and an air sampler based on filtration (AirPort MD8, Sartorius, Germany). Culture of airborne viable fungi was performed on malt extract agar (ME) and dichloran glycerol-18 (DG18) at 25 or 37 °C. CIP 10-M and AirPort MD8 were shown to display comparable performances but significant differences were observed between culture conditions for *Aspergillus* spp. ( $p < 0.01$ ), *Scopulariopsis* spp. ( $p = 0.02$ ) and unidentified molds ( $p < 0.01$ ).

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### 1. Introduction

Fungal elements represent a significant part of the biological contaminants that could be detected in air and many investigations demonstrated that exposure to airborne fungal particles can cause a variety of adverse health effects in humans and domestic animals (Fung and Hughson, 2003; Samson et al., 1994). In animal facilities, environmental conditions are favorable for the development of a wide variety of fungal species (Duchaine et al., 2000). This kind of contamination may cause health problems in workers (Chang et al., 2001; Cormier et al., 2000a; Reboux et al., 2006) and in animals, especially avian species (Lair-Fullerger et al., 2006).

In order to increase poultry production, domestic birds are usually reared in confined buildings with a densely stocked population. Fungi introduced with litter or feed can easily grow and sporulate under appropriate environmental conditions. Subsequently, spores or hyphae fragments can be aerosolized periodically with animal movements or air droughts (Lair-Fullerger et al., 2006).

The dynamic impact of airborne fungi on indoor agricultural air quality remains poorly understood. This is partly due to the lack of

standardized sampling methodology and the need for appropriate culture media and conditions adapted for surveys in poultry farms.

Many different designs of air sampler have evolved to match the different characteristics of the organisms and the forms of analysis to be applied. The devices allow various sized particles to be settled, impacted, filtered or impinged onto various substrates such as porous filters, agar media, adhesive films or into liquids (Thorne et al., 1992; Bex et al., 2003; Gangneux et al., 2006).

The objective of the present study was to assess the relative efficiencies of two air sampling methods and three culture conditions for the quantification of airborne culturable fungi in a poultry farmhouse in France. The effect of environmental parameters (temperature, relative humidity and animal density) on retrieval of airborne fungi was evaluated during a 15 week surveillance program. Two devices were used simultaneously—the CIP 10-M and the AirPort MD8, which allowed the collection of airborne particles in a liquid and on a gelatine membrane, respectively.

### 2. Materials and methods

#### 2.1. Sampling devices

Air samples were obtained using a CIP 10-M and the AirPort MD8 (Table 1).

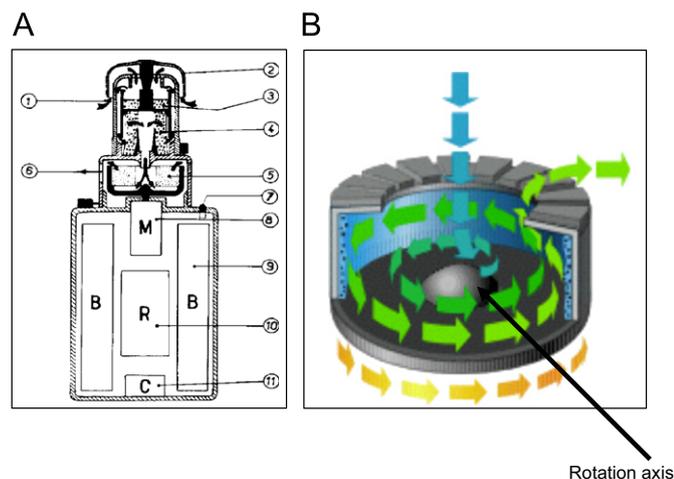
The CIP 10-M (*Captteur Individuel de Poussières Microbiologiques*, Arelco Company, Fontenay-sous-Bois, France) is an inhalable aerosol sampler (Courbon et al., 1988;

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**Table 1**  
Characteristics of two air samplers tested in present study.

Name	Manufacturer	Programmable (time/volume)	Flow rate (L min <sup>-1</sup> )	Sample collection	Autonomy (h)	Weight (kg)
CIP 10-M	Alreco, Fontenay-sous-Bois, France	No	10	Liquid	40	0.3
AirPort MD8	Sartorius, Goettingen, Germany	Yes	Adjustable (30, 40 and 50)	Gelatine membrane	4.5	2.5



**Fig. 1.** Diagram of rotating cup apparatus CIP 10-M (Arelco Company) (A); rotation of cup and air movement inside cup (B); 1—Air inlet; 2—inlet protector; 3—selector; 4—aerosol fraction; 5—rotative cup; 6—air outlet; 7—on/off diode; 8—motor; 9—batteries; 10—regulation; 11—recharging socket.

Görner et al., 2006). It was originally validated for the detection of non-culturable fungi of the genus *Pneumocystis* (Guillot et al., 1999). More recently, CIP 10-M has been used for the characterization of fungal aerocontamination by measurement of ergosterol in air (Robine et al., 2005) and by TTGE fingerprinting (Nieguitsila et al., 2007). The rotation of the cup at about 7000 rpm inside its housing maintains a flow rate of 10 L min<sup>-1</sup>. Airborne microorganisms are aspirated through an air inlet and enter the rotating cup axially (Fig. 1A). Then, the particles are driven by centrifugal force toward the liquid collection surface, maintained in a vertical position in the cup due to centrifugal force. Particles follow a helicoidal trajectory and are deposited in the sampling liquid (Fig. 1B). The configuration of the CIP 10-M air flow is supposed to cause minimal stress to the microorganisms. There is neither mechanical impaction nor pressure drop shock. The physical collection efficiency of CIP 10-M is superior to 50% for particles larger than 1.8 μm in aerodynamic diameter. The overall sampling efficiency of the sampler is a combination of the selection and collection efficiency (Görner et al., 2006). The collection efficiency decreases with decrease in particle size but is similar to those of many single-stage microbiological impactors (Fabriès et al., 2001). The CIP 10-M can be fit to three different particle selectors making possible the sampling of health-related aerosol fractions (inhalable, thoracic and respirable). In the present study, we selected the inhalable fraction.

The Airport MD8 sampler (Sartorius, Goettingen, Germany) is a filtration-based bioaerosol collector (Engelhart et al., 2007). The sampling head inserts directly in the pump unit without an interjacent flexible hose, and the flow rate is adjusted to 50 L min<sup>-1</sup>. The filter head contains a gelatine membrane (Sartorius AG, Biotechnology Division, Germany), which can be dissolved in distilled water at 37 °C (Parks et al., 1996). The filter maintains the viability of the collected microorganisms. High retention capacity of the filter is maintained even under extreme ambient conditions in terms of temperature and relative humidity (Sartorius application note). According to the manufacturer the retention rate is 99.9995% for *Bacillus sub. niger* spores. The Airport MD8 sampler was recently used for the characterization of fungal aerocontamination by D-HPLC fingerprinting (Nieguitsila et al., 2010).

## 2.2. Sampling site and procedure

The study was carried out in a 400 m<sup>2</sup> henhouse for 15 weeks. The building sheltered about 4300 broiler chickens with a static ventilation system. After 6 weeks, all the animals had free access to an outdoor area (2 m<sup>2</sup>/bird), which was a green pasture with some trees. Indoor litter consisted of fresh straw spread on the floor a few days before the arrival of one-day-old chicks. Extra straw was added when necessary.

The study was performed in spring 2007 in Burgundy, a French region with continental climate. Samples of 500 L of indoor air were simultaneously collected with the two devices, once a week, throughout the 13 weeks period of grow-out, the week before the arrival of the animals and the week after their departure to the slaughterhouse.

Air samplers distant of 3 m were placed at 0.5 m above the ground. With CIP 10-M, airborne particles were collected in 2 ml of distilled sterile water with 0.01% Tween 20. With AirPort MD8, airborne particles were filtered by the gelatine membrane, which was further dissolved in 10 mL of distilled sterile water at 37 °C. The sterility of the membranes was guaranteed by the manufacturer (Sartorius AG). However negative controls were included every week. A membrane (which was not previously used with the Airport MD8 air sampler) was dissolved in distilled water at 37 °C, seeded onto mycological media and incubated at 25 °C. The liquid used in CIP10-M was also tested for the presence of fungal contamination every week.

Indoor temperature, relative humidity (Testo SARL Forbach, France) and NH<sub>3</sub> concentration (Draeager, Germany) were recorded every week in the facility.

## 2.3. Culture media

The collection liquid (from CIP 10-M) and the dissolved gelatine membrane (from AirPort MD8) were diluted with distilled water at 1/10, 1/100 and 1/1000. Each dilution was vortexed for 30 s. A volume of 500 μL was then seeded onto mycological media in Petri dishes.

The medium ME was composed of malt extract, 20 g/L (Fluka, Biochemika); agar, 15 g/L (Labosi); peptone, 1 g/L (Difco); glucose, 20 g/L and chloramphenicol, 0.5 g/L. ME is extensively used in aerobiological studies as a broad spectrum medium for fungi collection and recommended by the American Conference of Governmental Industrial Hygienist (ACGIH; Burge et al., 1987).

The Petri dishes containing ME were incubated at 25 and 37 °C for 7 days and examined daily.

The medium DG18 was composed of glucose, 10 g/L; peptone, 5 g/L; NaH<sub>2</sub>PO<sub>4</sub>, 1 g/L; Mg<sub>2</sub>SO<sub>4</sub>, 0.5 g/L; dichloran, 0.002 g/L, agar, 15 g/L; glycerol, 220 g/L and chloramphenicol, 0.5 g/L (Hocking and Pitt, 1980). Glycerol is used as a suitable solution for the cultivation of many xerophilic fungi. Dichloran restricts the growth of fast-growing fungi (such as Mucorales) and limits the colony diameters of other species, and therefore facilitates the counting of the colonies. The Petri dishes containing DG18 were incubated at 25 °C for 7 days and examined daily.

## 2.4. Fungal identification

Molds were identified by their macroscopic and microscopic appearance after lactophenol cotton blue staining (de Hoog et al., 2000). Yeasts were subcultured and identified with the Api32C system (BioMerieux, Marcy l'Etoile France). For each positive sample, fungal contamination was estimated by counting the number of cfu/m<sup>3</sup>.

## 2.5. Statistical analysis

A repeated measure ANOVA was used to compare the number of colonies isolated with both devices under 3 different culture conditions, as well as to evaluate the effect of birds management conditions on the number of colonies. The dependent variable was the natural logarithm of the fungal concentrations (cfu/m<sup>3</sup>). Independent variables were the sampling device (2 modalities) and the culture condition (3 modalities) as well as the interaction between these two variables. Evaluation of the birds management conditions on fungal concentration was done by adding the corresponding variable (2 modalities according to outdoor access—yes or no) to the preceding model. We used software R (R Development Core Team, Austria, 2006, <http://www.R-projet.org>). A *p*-value of less than 0.05 was considered statistically significant.

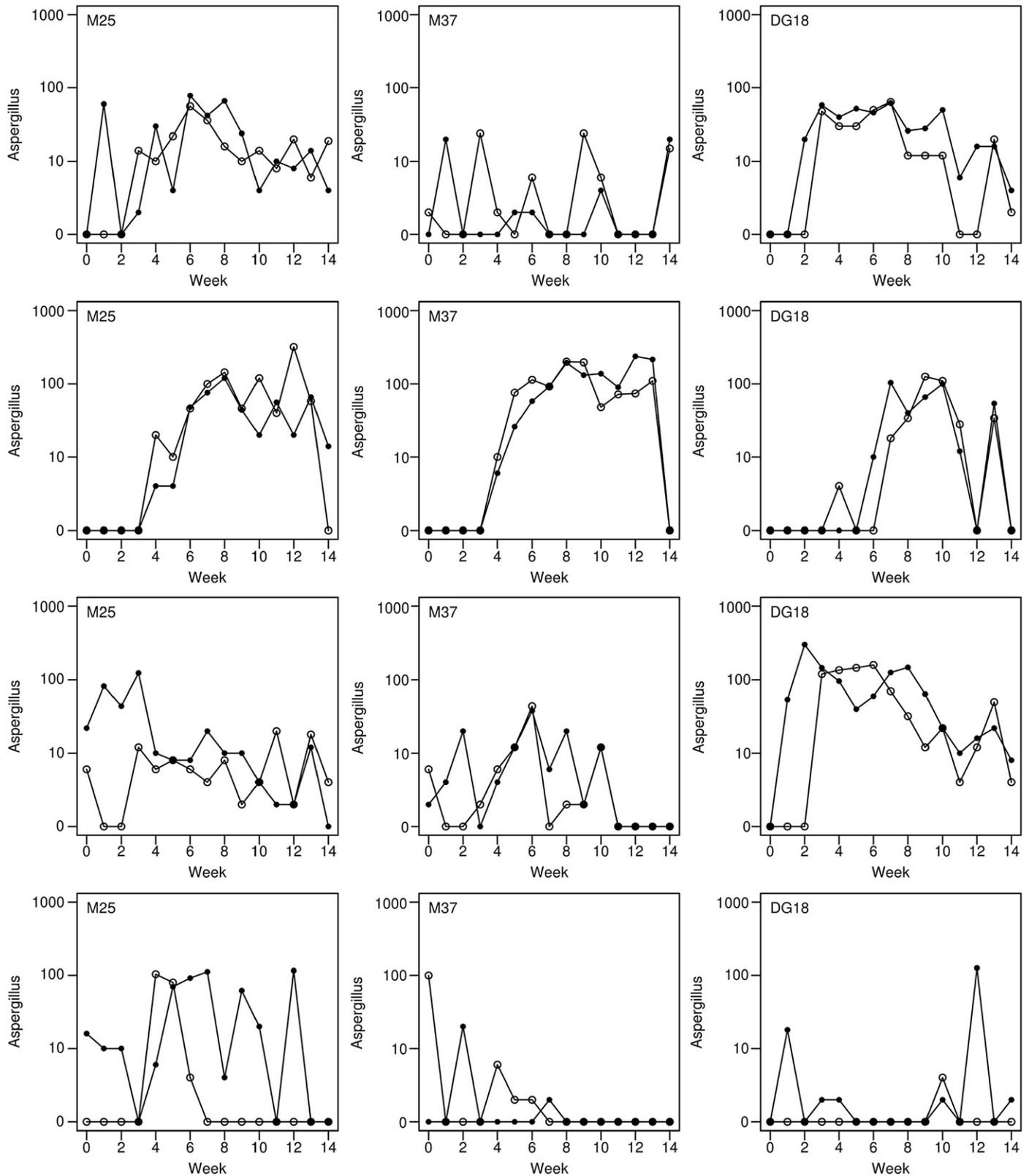
## 3. Results

Considering the whole sampling period, mean fungal concentrations with CIP 10-M and AirPort MD8 were 161.5 and 95.7 cfu/m<sup>3</sup>

on DG18 at 25 °C ( $n=15$ , standard deviations—121.6 and 78.2, respectively), 123.6 and 101 cfu/m<sup>3</sup> on ME at 25 °C ( $n=15$ , standard deviations—71.5 and 92.3, respectively), and 95.1 and 85.8 cfu/m<sup>3</sup> on ME at 37 °C ( $n=15$ , standard deviations—82.7 and 68.4, respectively).

Strong variations of cfu values occurred during the sampling period (Fig. 2).

Most fungal organisms could be identified at the level of the genus. Fungi most frequently recovered in all the samples belonged



**Fig. 2.** Values of colony forming unit throughout 15-week sampling period in poultry farmhouse. Each column corresponds to a specific culture condition (M25 = malt extract agar at 25 °C; M37 = malt extract agar at 37 °C and DG18 = dichloran glycerol-18 at 25 °C). Each line corresponds to a fungal type. Curves with points represent values obtained from air samples made with CIP 10-M and curves with circles represent values obtained from air samples made with AirPort MD8.

to the genera *Aspergillus* (including *Aspergillus fumigatus*), *Penicillium*, *Alternaria*, *Scopulariopsis* and *Cladosporium*.

Mucorales and yeasts were also regularly isolated (*Rhodoturula* spp., *Candida* spp. including *C. catenulate* and more seldom *C. albicans*). Several fungal organisms could not be identified with certainty either because they did not sporulate or because they form spores in a way that could not be associated with an already known group of fungi. These organisms were designated as “unidentified molds”.

### 3.1. Efficiency of two air samplers

Fig. 2 shows the evolution of cfu counts during the 15-week sampling period. All together the data obtained using CIP 10-M were comparable to the AirPort MD8 data. There was no significant difference between the efficiencies of the two air samplers except for unidentified molds ( $p=0.02$ ), which were more abundantly detected in air samples from CIP 10 than in those from Airport MD8 (Fig. 3). *Alternaria* spp., *Cladosporium* spp. and yeasts were more frequently detected with CIP 10-M but the difference was not significant.

### 3.2. Comparison of culture conditions

Culture conditions had a significant effect on fungi concentration for *Aspergillus* spp. ( $p < 0.0001$ ), *Scopulariopsis* spp. ( $p=0.0001$ ) and unidentified molds ( $p < 0.0001$ ). For *Aspergillus* spp., higher cfu values were obtained with DG18 and ME at 25 °C (30.28 and 23.06 cfu/m<sup>3</sup>, respectively) than with ME at 37 °C (3.20 cfu/m<sup>3</sup>). For *Scopulariopsis* spp., higher cfu values were obtained with ME at 37 °C (79.06 cfu/m<sup>3</sup>) than with DG18 (27.57 cfu/m<sup>3</sup>). For

unidentified molds, higher cfu values were obtained with DG18 at 25 °C (79.71 cfu/m<sup>3</sup>) than with ME at 25 °C (23.87 cfu/m<sup>3</sup>) or with ME at 37 °C (8.00 cfu/m<sup>3</sup>). *Alternaria* spp. were isolated on ME (except on week 1) and in samples from CIP 10-M, (except the week after the departure of the birds). Most of the yeasts were isolated on ME. Mucorales were detected only on ME during the 15-week sampling period.

There was no significant impact of NH<sub>3</sub>, ambient temperature or relative humidity on either global mycoflora or specific fungal groups concentration during the survey.

### 3.3. Effect of outdoor access on concentrations of airborne fungi

To evaluate the relationship between outdoor access and the concentrations of three types of fungi (*Aspergillus* spp., *Scopulariopsis* spp. and unidentified molds) a repeated measure ANOVA was performed adding birds management conditions as independent variable (Table 2). Significant differences were detected between the indoor period (from week 1 to 4) and the period of free outdoor access (from week 5 to the end). *Scopulariopsis* spp. ( $p < 0.0001$ ) and unidentified molds ( $p=0.01$ ) were more frequently isolated during the period of free outdoor access. There was no significant difference between the two periods for *Aspergillus* spp. ( $p=0.12$ ).

## 4. Discussion

To assess the level of exposure to airborne fungal organisms, many investigations have been made in different environments including hospitals (Nesa et al., 2001; Wu et al., 2000), buildings (Shelton et al., 2002; Verhoeff et al., 1990) and agricultural

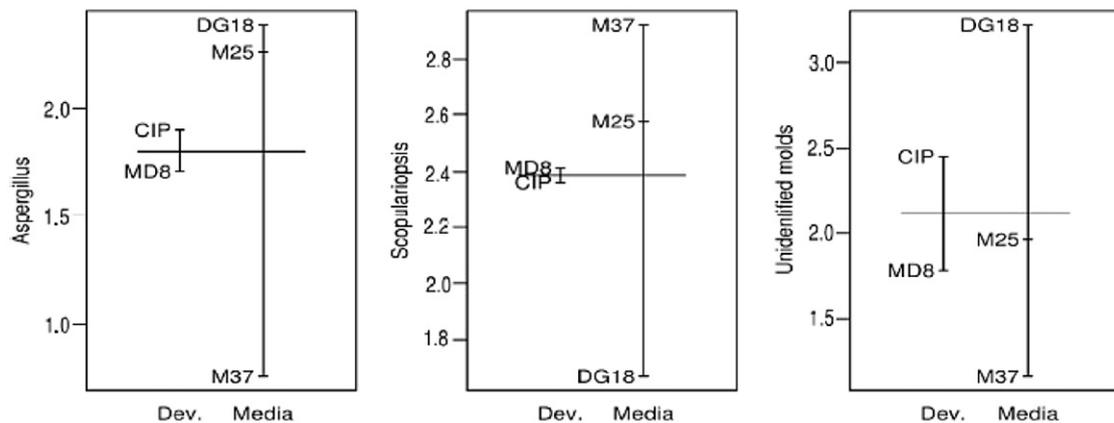


Fig. 3. Comparison of mean concentrations (cfu/m<sup>3</sup>) of fungal genera or groups whose numerations differed significantly dependent on devices (Dev.) and culture conditions. CIP is CIP 10-M sampler; MD8 is AirPort MD8 sampler and culture conditions are represented by M25 = malt extract at 25 °C; M37 = malt extract at 37 °C and DG18 = dichloran glycerol-18 at 25 °C.

Table 2

Arithmetic mean concentrations (cfu/m<sup>3</sup>) of *Aspergillus* spp., *Scopulariopsis* spp. and unidentified molds in air. Two periods were defined—from week 1 to week 5, birds had no outdoor access; from week 6 to week 13, animals had a free outdoor access.

	Culture conditions*	<i>Aspergillus</i> spp.		<i>Scopulariopsis</i> spp.		Unidentified molds	
		CIP 10-M	AirPort MD8	CIP 10-M	AirPort MD8	CIP 10-M	AirPort MD8
No outdoor access (weeks 1–5); sample size=5	ME 25 °C	19.20	9.20	1.60	6.00	53.60	5.20
	ME 37 °C	4.40	5.20	6.40	17.20	8.00	4.00
	DG18 25 °C	34.00	21.60	0.00	0.80	128.00	80.40
Outdoor access (weeks 6–13); sample size=8	ME 25 °C	30.75	20.75	56.25	109.00	8.50	8.00
	ME 37 °C	0.75	4.50	144.25	113.25	9.75	7.50
	DG18 25 °C	31.25	21.25	48.25	43.75	58.50	45.25
		$p > 0.05$		$p < 0.01$		$p < 0.01$	

environments (Adhikari et al., 2004; Chang et al., 2001; Cormier et al., 2000b; Duchaine et al., 2000; Lair-Fullerger et al., 2006; Lee et al., 2006; Lugauskas et al., 2004; Reboux et al., 2006; Thorne et al., 2009). However, different procedures of air sampling and analysis have been used and the results obtained in these investigations are hardly comparable. Several investigations were conducted for the assessment of fungal contamination in barns housing swine (Chang et al., 2001; Cormier et al., 2000a; Jo and Kang, 2005; Létourneau et al., 2010; Thorne et al., 1992, 2009). Only a few were conducted in poultry facilities (Lair-Fullerger et al., 2006; Gemeinhardt and Wallenstein, 1985; Jo and Kang, 2005; Lugauskas et al., 2004). Adhikari et al. (2004) suggested that the contrasting observations of airborne fungal concentrations in agricultural environment of different countries could be attributed to the different structure of agricultural confinements, diverse local sources for fungal growth in different climates, environmental parameters, cleaning and animal handling activity and maintenance of environment.

Most investigations on indoor air were conducted with culture-based methods, but insufficient attention was generally given to four important issues—sampler performance, temporal variability, culture media and accurate identification. Several air samplers are currently marketed and are based on different physical principles (mainly impaction, centrifugal acceleration or filtration). Impactors remain the most widely used type of sampler. In 1989, the American Conference of Governmental Industrial Hygienists (ACGIH) outlined several sampling methodologies for indoor monitoring but did not report the comparability of data derived from these various methods (ACGIH, 1989). In the present study, the choice of samplers was based on the following criteria: commercial availability, presence of a battery giving at least 1 h of autonomy, weight, simplicity of use under difficult conditions (a farmhouse with a large number of living animals), limited stress to the microorganisms and the possibility to collect large particles (like fungal spores). According to these criteria we selected a rotating cup sampler (CIP 10-M) and a device using air filtration (AirPort MD8).

The statistical analysis showed that the performance of the CIP 10-M and the AirPort MD8 regarding the collection of culturable fungi was comparable. This result may look surprising because the two devices do not collect air particles in the same way and because the flow rate of the two devices is very different ( $50 \text{ L min}^{-1}$  for MD8 versus  $10 \text{ L min}^{-1}$  for CIP 10-M).

Regarding culture conditions, a comparison of cfu values for total fungi and individual fungal types was performed. ME agar allowed the growth of many different fungal organisms. ME was extensively used in aerobiological studies and is still recommended by the ACGIH for detection and enumeration of fungi in indoor environments (Burge et al., 1987). DG18 is more seldom used in aerobiological studies. It was first developed for the enumeration of moderately xerophilic molds and osmophilic yeasts in food (Hocking and Pitt, 1980). So far, only one field study adopted this medium for environmental airborne fungi sampling (Verhoeff et al., 1990), and two methodological studies suggested that DG18 could be an alternative choice for easier colony counting and higher yield of cfu and types of fungi (Verhoeff et al., 1990; Smid et al., 1989). The study conducted by Wu et al. (2000) demonstrated that DG18 was superior to ME for yeasts enumeration. Our observations were not in accordance with the latter study as yeasts were recovered more frequently on ME than on DG18 (except on week 12). DG18 seems to limit the growth of Mucorales as already suggested by Hocking and Pitt (1980).

The fungal organisms identified in the present study had already been isolated from confined agricultural environments (Adhikari et al., 2004; Cormier et al., 2000a,b; Duchaine et al., 2000; Lugauskas et al., 2004; Reboux et al., 2006; Thorne et al., 2009). During an investigation in a poultry facility in Lithuania, 31 species attributed to 13 fungal

genera were isolated (Lugauskas et al., 2004). *Aspergillus oryzae* and *A. nidulans* prevailed and made up 15.1% and 9.7% of all identified isolates, respectively. The genus *Penicillium* was represented by 12 different species. Mucorales were also isolated. In the air from poultry facilities in Germany, Gemeinhardt and Wallenstein (1985) detected a large number of fungal organisms including many *Penicillium* spp. and *Scopulariopsis brevicaulis*.

Concentration for each individual fungal type varied according to the culture conditions. Both DG18 (at  $25^\circ\text{C}$ ) and ME allowed the detection of *Aspergillus* spp. ME was very efficient for the isolation of *Scopulariopsis* spp. whereas DG18 allowed the growth of unidentified molds. In an investigation conducted in human habitats (Verhoeff et al., 1990), the use of DG18 yielded the highest fungal concentrations. Another study performed in a hospital (Wu et al., 2000) showed that DG18 allowed the isolation of more fungal types than ME. Chao et al. (2002) found a better recovery for the genus *Aspergillus* on DG18 than on ME medium. As a consequence, the use of different mycological media may be recommended in order to improve the accuracy of the evaluation of fungal aerocontamination in a heavily contaminated place (like an animal facility).

The concentration of *Scopulariopsis* was significantly lower in the first four weeks (when birds lived only indoors) in comparison with the last eight weeks of rearing (when they had a free outdoor access). On the contrary, the number of unidentified molds was significantly higher in the first four weeks than in the last part of rearing. These results demonstrated that outdoor access yielded significant variations in indoor airborne fungal concentrations.

## 5. Conclusion

The dynamic impact of airborne fungi on indoor air quality in poultry farms remains poorly understood. In the present study, we demonstrated that two air samplers (CIP 10-M and AirPort MD8) displayed comparable performances for the detection of culturable fungi in a farm in France. Fungi most frequently recovered during the 15-week sampling period belonged to the genera *Aspergillus*, *Penicillium*, *Alternaria*, *Scopulariopsis* and *Cladosporium*. Significant differences were observed between culture conditions for *Aspergillus* spp., *Scopulariopsis* spp. and unidentified molds.

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