

Assessment of the aerosolization potential for fungal spores in moldy homes

Abstract The airborne fungal concentration measured with air samplers during specific time intervals may not adequately represent the indoor air quality because of the sporadic nature of spore release from sources. The conventional source evaluation (e.g. swab and tape sampling) characterizes the mold source but does not relate to the fraction of spores that can be aerosolized from a contaminated material. As an alternative to these methods, we have recently developed and laboratory-tested a novel Fungal Spore Source Strength Tester (FSSST). It allows assessing the potential of aerosolization of fungal spores from contaminated surfaces under the most favorable release conditions. In this study, the FSSST was used to characterize the release of spores from four building materials in mold-problem homes. The spores of different species were efficiently aerosolized by the FSSST, exhibiting a total spore release rate ranging approximately from 10^2 to 10^3 cm^2/min . For all tested materials, < 2% of the spores on the contaminated surface were released during the tests. The airborne spore concentration estimated from the release rate data was found in most cases to be significantly greater than the concentration actually measured in these environments with simultaneous air sampling. The results suggest that the FSSST can be used for the assessment of maximum potential exposure to airborne spores released from identified sources in homes.

**S. K. Sivasubramani,
R. T. Niemeier, T. Reponen,
S. A. Grinshpun**

Center for Health-Related Aerosol Studies, Department of Environmental Health, University of Cincinnati, Cincinnati, OH, USA

Key words: Fungi; Moldy buildings; Spore release; Aerosol sampling; Source strength.

Sergey A. Grinshpun
Center for Health-Related Aerosol Studies, Department of Environmental Health, University of Cincinnati, Cincinnati, OH 45267-0056, USA
Fax: 1 513 558 2263
e-mail: sergey.grinshpun@uc.edu

Received for review 5 January 2004. Accepted for publication 30 March 2004.
© Indoor Air (2004)

Practical Implications

A recently developed FSSST was found to be suitable to measure the aerosolization potential of indoor fungal sources at the most favorable release conditions. The FSSST generates the data that allows assessing the strength of mold sources in homes with respect to their maximum ability to contaminate indoor air with fungi. The novel approach bridges two conventional methods, the air sampling and the direct source evaluation (e.g. swab sampling), thus providing a better representation of the airborne fungal exposure than these methods individually. The device prototype can be used for evaluating the effectiveness of environmental interventions by taking samples before and after the intervention. As a broader application, the FSSST can be utilized for assessing the release of various hazardous biological and non-biological particles from contaminated surfaces.

Introduction

Numerous health effects in homes, schools, and offices have been attributed to the fungal growth resulting from water damage or improper humidity in buildings (Dales et al., 1991a; Lacey and Crook, 1998; Wickman et al., 1992). The exposure to mold and dampness has been associated with respiratory symptoms (Dales et al., 1991b) and may particularly increase the risk of adverse respiratory health effects (Peat et al., 1998; Verhoeff and Burge, 1997).

The fraction of buildings with mold-contamination in the United States and Canada is about 36%, according to Spengler et al. (1993). Brunekreef et al. (1989) have reported that 20–40% of homes in Northern Europe and North America have mold problems.

In the Netherlands and Finland, the mold and dampness in buildings were reported as 15 and 24%, respectively (Pirhonen et al., 1996; Verhoeff et al., 1990). The age of a building is an important factor affecting the fungal spore concentration in indoor air (Pasanen et al., 1992; Rand, 1999). Modern buildings are constructed with various types of materials that provide ecological niches with varying nutritional and temperature conditions. A variety of interactions between microorganisms may occur under these conditions. While practically all building materials can serve as a substrate for fungal growth when excess moisture is present, the growth and aerosolization rates may differ for different materials. The fungal growth mainly depends on the nutrient availability, alkalinity, porosity, and the water activity of the material. Water

activity is defined as the amount of free water in the material available for microbial growth and depends on the moisture absorbing potential of the growth material (Flannigan and Morey, 1996).

Wood and wood composites as well as materials with high starch content are capable of supporting mold growth at the lowest values of water activity (Hukka and Viitanen, 1999; Nielsen et al., 2000; Viitanen and Bjurman, 1995). Plasterboard reinforced with cardboard and paper fibers, as well as inorganic materials coated with paint or treated with additives that offer an easily degradable carbon source, are excellent substrate for molds. However, they support fungal growth only at relatively high water activity (Chang et al., 1995, 1996). Other inorganic materials with traces of organic ones seem to be able to support growth at very high water activity (Nielsen, 2003).

Variations in indoor humidity and temperature exert a profound influence on mold growth (Viitanen and Bjurman, 1995). The local differences in ventilation and surface temperature can generate microclimates with very high water activity. For this reason, a measurement of indoor relative humidity may not be sufficient to predict mold problems (Becker, 1984; Grant et al., 1989; Gravesen et al., 1999; Hukka and Viitanen, 1999). Water activity is the most important factor in determining whether or not mold growth is initiated on building materials (Ayerst, 1966; Galloway, 1935; Hukka and Viitanen, 1999; Rowan et al., 1999; Scott, 1957).

The release of fungal spores from their source is driven by the energy from external sources and may be significantly affected by environmental factors. The aerosolization caused by air currents seems to be the most prevalent mechanism for indoor fungi (Gregory, 1973; Madelin, 1994). For example, for *Aspergillus* and *Penicillium*, the air currents have been indicated as the principle physical factor causing the spore detachment and dispersion in indoor environments (Burnett, 1976). Zoberi (1961) found that the release of spores of different fungal species from agar surface is mainly a function of air velocity so that the velocity increase causes increase in the spore release rate. Pasanen et al. (1991) reported that the air velocity required for the spore release was dependent on the fungal type. Kildesø et al. (2003) demonstrated that the release of spores from the wet wallpapered gypsum board differs for different fungi under identical conditions. We found that the fungal spore release was affected by the air velocity above the surface, texture of the surface, and vibration of the contaminated material (Görny et al., 2001).

Air sampling and testing of building materials are often performed to identify the agents that are potentially responsible for health problems and to determine the level of exposure to these agents (Beguin and Nolard, 1994; DeKoster and Thorne, 1995; Icenhour

and Levetin, 1997; Macher and Huang, 1991). In principle, the air sampling should be the most representative of human respiratory exposure (Burge, 1995). However, this method is often not representative for assessing a long-term exposure to airborne fungal spores because the sampling period is limited and the spore aerosolization is sporadic. In case the air samples are analyzed through the culture-based enumeration, low viability/culturability may be a reason that some species are not detected. The above factors as well as the lack of knowledge about the actual mechanisms of diseases are essentially responsible for a poor association between the health effects and the exposure assessment data generated from the analysis of air samples. As an alternative to the air sampling, some exposure studies are carried out through the direct evaluation of fungal sources (Flappan et al., 1999; Meyer et al., 2004). The currently available techniques such as bulk sampling, surface sampling and dust sampling allow the investigators to collect extensive information about the source, but cannot measure its aerosolization potential and, therefore, may not adequately represent the aerosol exposure. For instance, Chew et al. (2003) reported the air and dust represent different types of potential fungal exposures.

To bridge two conventional methods (the air sampling and the direct source evaluation), the Fungal Spore Source Strength Tester (FSSST) was recently developed (Grinshpun et al., 2002). The new method, in which the spores are aerosolized from the source into a small chamber and immediately collected from it with a bioaerosol sampler, was recently evaluated under controlled laboratory conditions (Sivasubramani et al., 2003). The FSSST prototype was found suitable for assessing the aerosolization potential of the fungal spores from the growth source. This non-destructive assessment of fungal contamination on building materials seems to be an important additional advantage of the FSSST. The objective of this study was to use the FSSST prototype for determining the rate and efficiency of fungal spore release from different contaminated building materials in mold-problem homes.

Materials and methods

Four residential homes with visibly mold-contaminated surfaces, including concrete, painted dry wall, particleboard and wooden joist, were selected in the Greater Cincinnati metropolitan area. Because of its climate conditions and frequent flooding, the area has a considerable number of water-damaged homes and a very high occurrence of respiratory allergies and asthma, especially in children. One mold-damaged room was tested in each home. The main criterion was that the room had at least 0.5 m² of the visible moldy surface with a uniform contamination to allow for testing with the swab method and by the FSSST. The

uniformity of the mold growth was checked with a hand-held magnifier lens. The test rooms were of about the same size (the room volumes ranged from 107 to 121 m³).

Prior to the experiment, the indoor temperature and relative humidity were measured with a traceable humidity/temperature pen (Fisher Scientific Company, Pittsburgh, PA, USA). The surface moisture content of the test surface was measured with a Protimeter (GE Protimeter, Wilmington, MA, USA) and expressed as a percentage of the mass of water in a given volume of a material, (wet mass – dry mass) × 100/(dry mass). For a specific material, this percentage is calculated as a wood-equivalent value. Each building material, tested in this study, was characterized with respect to the initial spore contamination using the swab sampling method. A sample of approximately 1 cm² was taken with a sterile wet swab from four different places, randomly chosen on the test surface. Each sample was suspended in 20 ml of de-ionized, sterilized water with 0.05% Tween 80 (Sigma chemicals Co., St Louis, MO, USA) in a test tube. The swab sample suspensions were vortexed. Three milliliter of suspension was vacuum filtered onto a 13-mm mixed cellulose ester filter (0.8 μm pore size). The spore-containing filters were dried by placing them in sterile Petri plates at room temperature for 2 h. Dry filters were placed on a glass slide and cleared by acetone vapor utilizing a modified instant acetone-vaporizing unit (model Quickfix, Environmental Monitoring Systems, Charleston, SC, USA). A 25 × 25 mm cover glass was mounted on the slide using glycerin jelly (Gelatin: 20 g, Phenol crystals: 2.4 g, Glycerol: 60 ml, Water: 70 ml). The spores on the slide were counted by using a light microscope (model Leitz Laborlux S, Leica Mikroskopie und Systeme GmbH, Germany, available through W. Nuhsbaum, Inc., IL, USA) at a 400× magnification. For slides with a dense spore deposit (> 50 spores per microscopic field), the spores were enumerated in 20 microscopic fields; and the slides with sparse deposit (< 50 spores per field), 40 microscopic fields counted. The total spore count and the count of individual spore types were performed on each sample. The initial spore surface density, N_S , was determined based on the number of spores counted on a 1-cm² area from which the swab sample was taken (N_S was calculated for both the total and spore-type-specific counts).

Following the above-described direct source evaluation, the FSSST was applied to the test surface. The device is schematically shown in Figure 1. It consists of a cup like aerosolization chamber, which is applied to and held against the mold-contaminated surface. A push vacuum pump produces the airflow Q_{IN}^{FSSST} that passes through the HEPA filter (1244 HEPA capsule filter, PALL Gelman Laboratory, Ann Arbor, MI, USA). The flow Q_{IN}^{FSSST} is directed through 112-orifice stage at the bottom of the device

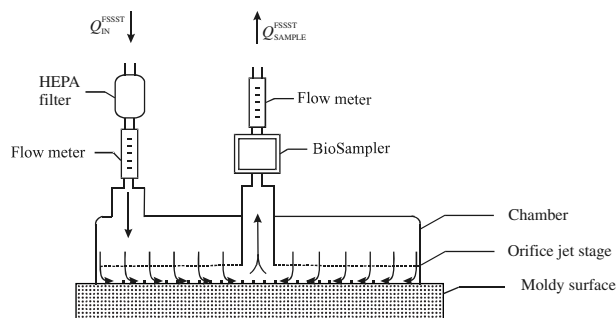


Fig. 1 Fungal spore source strength tester

creating air jets towards the contaminated surface. The air jet velocity that is approximately 13.6 m/s at the orifice decreases considerably towards the contaminated surface. The fungal spores, aerosolized by these air jets, are collected into the BioSampler (SKC Inc., Eighty Four, PA, USA). The sampler is located at the outlet and operated by a pull vacuum pump at a flow rate of $Q_{IN}^{FSSST} = 12.5$ l/min. The collection vessel is filled with a 20 ml suspension of de-ionized, sterilized water mixed with 0.05% Tween 80. The flow rate balance is set so that the Q_{IN}^{FSSST} is slightly lower than the Q_{IN}^{FSSST} ($\Delta = 1.0$ l/min). As a result, no fungal spore contamination occurs in the ambient air during the FSSST operation. Some spores may penetrate into the aerosol chamber from the outside air environment but their number is rather low compared with the number of spores released from the source because of the difference in (i) flow rates ($\Delta \ll Q_{IN}^{FSSST}$) and (ii) micro environmental fungal concentration (air < mold source). The airflows at the FSSST's inlet and outlet are controlled with flow meters (model 2A17, Key Instruments, Trevose, PA, USA) calibrated with a Buck calibrator (A.P. Buck, Orlando, FL, USA).

The FSSST was applied to the mold-contaminated surface during four time intervals: $t_{FSSST} = 5, 10, 15,$ and 20 min. Thus, four samples were collected on each surface. The device was thoroughly cleaned between the tests with 70% ethyl alcohol, and a separate sterile BioSampler was used for each test. Simultaneously with the use of the source tester, four indoor air samples were collected by the BioSampler ($Q_{AIR}^{SAMPLE} 12.5$ l/min) at about 2 m away from the source using the same sampling time intervals as for the FSSST. After the sampling, the collection fluid of each BioSampler (attached to the FSSST and acting as a stationary air sampler) was filtered through a mixed cellulose ester filter, which was subsequently cleared by acetone vapor and analyzed under the microscope as described above.

The spore release rate (R , per square centimeter and per minute), defined as the number of airborne spores aerosolized from 1 cm² of the test surface during one minute of the FSSST application, was determined as:

$$R = \frac{N_{\text{FSSST}}}{A \times t_{\text{FSSST}}} \quad (1)$$

where N_{FSSST} is the number of spores obtained in the BioSampler attached to the source tester, and A is the surface area subjected to the source testing ($A \approx 140 \text{ cm}^2$). The airborne spore concentration was determined as

$$C_{\text{AIR}}^{\text{MEASURED}} = \frac{N_{\text{AIR}}}{Q_{\text{SAMPLE}}^{\text{AIR}} \times t} \quad (2)$$

where N_{AIR} is the number of spores obtained in the BioSampler operating as a stationary air sampler and t is the air sampling time. The measured spore release rate, R , can be used to assess the ‘worst-case’ scenario by estimating the airborne spore concentration as

$$C_{\text{AIR}}^{\text{ESTIMATED}} = \frac{R \times A_{\text{CONTAMINATION}} \times t_{\text{FSSST}}}{V_{\text{ROOM}}} \quad (3)$$

where $A_{\text{CONTAMINATION}}$ is the area of the contaminated surface and V_{ROOM} is the volume of the room. This assumes that (i) t_{FSSST} is sufficiently long to expect that most of the spores, which are ready to release, will become airborne, and (ii) no reduction of the aerosol concentration because of gravitational settling and other mechanisms is considered in this ‘worst-case’ scenario.

The relative efficiency of spore release [E_{R} (%)] was defined as the number of spores released from 1 cm^2 during the time of the FSSST’s application to the initial number of spores on the area of 1 cm^2 determined by the swab sampling method. The E_{R} values were calculated as:

$$E_{\text{R}} = \frac{R \times t_{\text{FSSST}}}{N_{\text{S}}} \times 100\% \quad (4)$$

Results

The highest total initial spore surface density, $N_{\text{S}} = (19.2 \pm 3.7) \times 10^5/\text{cm}^2$, occurred on the wooden joist, followed by particleboard $[(14.6 \pm 5.9) \times 10^5/\text{cm}^2]$, painted drywall $[(6.9 \pm 2.7) \times 10^5/\text{cm}^2]$, and concrete $[(3.6 \pm 0.6) \times 10^5/\text{cm}^2]$. In a wood-based material, readily available nutritional condition and the capillary action of water absorption (Pasanen et al., 2000) are likely to facilitate considerable fungal growth.

The identification analysis of swab samples, taken from the four tested building materials, revealed six spore types. The *Aspergillus/Penicillium* group was detected on all tested materials, except the wooden joist. *Chaetomium* spores were detected on painted drywall and particleboard. Spores of *Cladosporium* were found on concrete, painted drywall and wooden joist. Basidiospores and *Stachybotrys* were found only on painted drywall. The greatest number of fungal

types was identified in samples taken from the painted drywall. The following air temperature and relative humidity levels were recorded: approximately 21°C and 40% in homes where the particleboard and wooden joist were tested, 23°C and 20% in the home with mold-contaminated painted drywall, and 17°C and 69% in the home with moldy concrete surface. The surface moisture content observed in the tested materials was within a relatively narrow range of 9–15%.

The airborne concentrations of fungal spores observed in the tested homes ranged approximately from 10^4 to $10^5/\text{m}^3$. It was higher in the homes where mold-contaminated concrete and painted dry wall were tested [$C_{\text{AIR}}^{\text{MEASURED}} = (1.7 \pm 4.3) \times 10^4/\text{m}^3$ and $(10.8 \pm 0.6) \times 10^4/\text{m}^3$, respectively] and lower in the homes with particleboard and wooden joist [$C_{\text{AIR}}^{\text{MEASURED}} = (1.7 \pm 0.6) \times 10^4/\text{m}^3$ and $(1.4 \pm 0.2) \times 10^4/\text{m}^3$, respectively]. These airborne spore concentrations are of the same order of magnitude as those reported by Rautiala et al. (1996) from their aerosol measurements performed in moldy buildings.

All the fungal spore types identified from the swab samples were also found in the air samples. In addition, the air sample analysis revealed some fungal types that were not found in the swab samples, suggesting the influence of outdoor sources or/and non-identified indoor sources. Hyvärinen et al. (1993) also observed some fungal genera in air samples while these genera were not found in the surface samples.

Figure 2 shows the spore release rates obtained with the FSSST for each building material, when the source tester was applied during 5, 10, 15, and 20 min. It is seen from Figure 2 that the spores were efficiently aerosolized by the FSSST from all four tested materials with a total spore release rate ranging approximately from 10^2 to $10^3/\text{cm}^2/\text{min}$. While the average R -values (data not shown on Figure 2) were somewhat higher

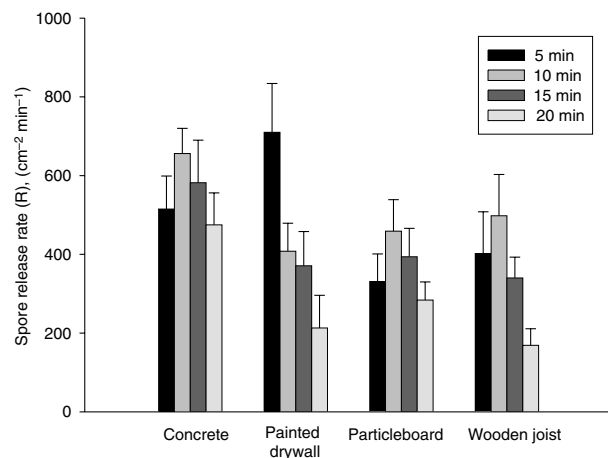


Fig. 2 Spore release rate for different contaminated surfaces and time of the FSSST application. Each R -value represents a single-test measurement and the error bars represent standard deviation of spore counts conducted on at least 20 microscopic fields

for concrete and painted dry wall than for the particleboard and wooden joist (following the initial spore surface densities, N_S), the material type had no dominant effect on the release rate. This can be attributed to the similarity in the physical properties of the four tested substrates (smooth and essentially non-porous materials). Other smooth non-porous materials, such as gypsum board release spores at about the same rate, while some porous substrates, such as ceiling tiles, may exhibit rates as high as approximately $10^4/\text{cm}^2/\text{min}$ (Sivasubramani et al., 2003). Figure 2 shows that the most intense spore release occurred during the first 10 min (concrete, particleboard, and wooden joist) or the first 5 min (painted drywall) of the 20-min FSSST application. A higher temperature and especially lower relative humidity recorded in the home with mold-contaminated drywalls suggest that fungal spores in that environment were easier to release (perhaps, in a greater extent than in other tested environments) (Foarde et al., 1999; Pasanen et al., 1991). Therefore, the majority of spores aerosolized during the first 5 min. For all the four tested materials, the spore release was observed continuously during the 20-min experiment and the release rate was significantly greater during the first 10-min than during the second 10-min of the 20-min interval. This finding confirms the results of our earlier laboratory study (Sivasubramani et al., 2003) performed with ceiling tiles, gypsum board, and agar artificially contaminated with *Aspergillus versicolor*. Similarly, Górný et al. (2001) reported that about 71–88% of the spores were released during the first 10-min of the 30 min experiment. The most matured spores are released within the first few minutes of the application of the external forces (in this case, air current) whereas longer exposure time is needed for the less matured spores.

The microscopic analysis showed that the spore types in the FSSST samples matched the ones identified in the swab samples on the respective contaminated surfaces. Interestingly, the FSSST samples revealed only the same spore types that were in the respective swab samples. This indicates that the chances of contamination of FSSST samples from the ambient air are negligible. At the same time, the ambient air samples had several spore types that were not identified in FSSST or swab samples. This demonstrates that the FSSST samples are more representative of the fungal source than the air samples taken in the vicinity of the source. In contrast to the traditional swab sampling method, the FSSST allows assessing the potential release of spores from the source in the worst-case scenario, which consequently provides the data for estimating the maximum plausible indoor concentration of spores.

Like for the total spore release, the release rate of *Aspergillus/Penicillium* spores was highest during the

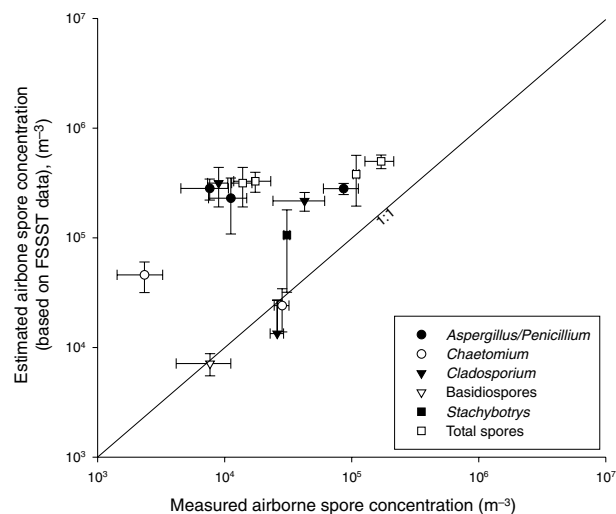


Fig. 3 The estimated airborne spore concentration vs. measured airborne spore concentration. Error bars indicate the standard deviation of four tests

first 10 min for concrete, particleboard, and wooden joist, and during the first 5 min for the painted drywall. The *Cladosporium* spores were released from concrete and wooden joist primarily during the first 10-min interval. No *Cladosporium* spores were detected on the FSSST samples taken from the particleboard. The swab sample analysis also revealed the absence of *Cladosporium* spores on this surface. Few *Cladosporium* spores were found to have released from the painted drywall. *Chaetomium* spores were aerosolized from the painted drywall and particleboard primarily during the first 5–10 min. *Stachybotrys* spores were detected only on the FSSST samples taken from the painted drywall, and the release rate of this spore type showed high variability from one test to another (Figure 3). As the *Stachybotrys* considered to be a wet spore type fungus (Duchaine and Mériaux, 2001), the local variation of the moisture level inside the fungal colony may have a great influence on the spore release into the air environment.

Table 1 shows the relative efficiency of the spore release. This category allowed us to relate the release rate (measured from the 10-min FSSST samples) to the initial spore surface density (determined from swab samples). Consequently, we compare the spore release from different materials having different initial spore surface density. The E_R -values are listed for the total spore count and for individual spore types. The data demonstrate that only a small fraction (not exceeding several percent) of the spores present in the substrate are aerosolized by the FSSST, although it creates very favorable aerosolization conditions. This finding agrees well with the data reported in our recent laboratory studies (Sivasubramani et al., 2002, 2003), in which low E_R -values were reported for other substrates. For the total spore count, the highest release efficiency was

Table 1 Relative efficiency of spore release

Building material	Spore type	Relative efficiency (%)
Concrete	Total spores	1.56 ± 0.22
	<i>Aspergillus/Penicillium</i>	1.48 ± 0.17
	<i>Cladosporium</i>	1.68 ± 0.32
Painted drywall	Total spores	0.62 ± 0.30
	<i>Aspergillus/Penicillium</i>	2.30 ± 1.22
	<i>Chaetomium</i>	0.12 ± 0.05
	<i>Cladosporium</i>	0.28 ± 0.29
	Basidiospores	0.28 ± 0.06
	<i>Stachybotrys</i>	0.45 ± 0.31
Particleboard	Total spores	0.28 ± 0.11
	<i>Aspergillus/Penicillium</i>	1.51 ± 0.62
	<i>Chaetomium</i>	0.05 ± 0.01
Wooden joist	Total spores	0.18 ± 0.07
	<i>Cladosporium</i>	0.18 ± 0.07

The data shows the average and standard deviation values of four tests.

found for concrete surfaces followed by painted drywall, particleboard and wooden joist. As described above, the spore release rate did not show a considerable dependence on the substrate material, which was attributed to the similar physical properties of the test materials. Therefore, the relative efficiency of spore release was greater for the substrate that had a lower initial spore surface density. Thus, the materials are in reverse order with respect to E_R (total spores) comparing to their N_S : the highest E_R was detected for concrete and the lowest for wooden joist. For the *Aspergillus/Penicillium* group, $E_R = 2.30 \pm 1.22\%$ for the painted drywall and $1.48 \pm 0.17\%$ for concrete, and the particleboard showed only $1.51 \pm 0.62\%$. Our laboratory study (Sivasubramani et al., 2003) of *Aspergillus/Penicillium* spore release from the naturally contaminated gypsum board showed $E_R = 1.39 \pm 0.85\%$, which is comparable with the data found in this study for all three materials on which *Aspergillus/Penicillium* spore growth was detected. Generally, the E_R -values may exhibit dependence on the substrate for the same fungal species, if the physical properties (the surface smoothness, porosity, etc.) are distinctly different. For example, the relative efficiency of release of *Aspergillus versicolor* spores ranged from $0.002 \pm 0.001\%$ (for the laboratory-inoculated agar) to $9.2 \pm 1.0\%$ (for the laboratory-inoculated gypsum board) (Sivasubramani et al., 2003). The relative efficiency of release of *Cladosporium* spores was found to be higher on the concrete substrate ($1.68 \pm 0.32\%$) than on painted dry wall ($0.28 \pm 0.29\%$) and wooden joist ($0.18 \pm 0.07\%$), following the trend observed for the total spore count. Overall, the concrete surface and painted drywall provide low nutritional condition and high resistance to the fungal growth. As a result, the spores grown on these surfaces are less bounded with conidiophores (and thus ready to release) than those grown on the particleboard and wooden joist. The data show that the efficiency of spore release may vary with

the fungal type, although it remained below 1% for all the identified types, except for *Aspergillus/Penicillium* and *Cladosporium*.

As the FSSST simulates the conditions most favorable for the spore aerosolization, we utilized the collected data for assessing the fungal spore concentration in indoor air, assuming that all spores were released from identified indoor sources. The assessment was conducted for a typical room with approximately 1% of the total surface area heavily contaminated with fungi. The following dimensions were applied: $V_{\text{ROOM}} = 7 \times 4 \times 4 \text{ m} = 112 \text{ m}^3$ (which is an average volume of the four test rooms selected for this study); $A_{\text{CONTAMINATION}} = 1 \text{ m}^2$. The airborne spore concentration was estimated assuming that the spores were released from the growth substrate at a rate as high as the one provided by the FSSST during 10 min (t_{FSSST}) (defined as a worst-case scenario). The estimated concentrations of different fungi, identified in four homes with different mold-contaminated substrates, are plotted in Figure 3 against the airborne spore concentrations, which were actually measured in the respective homes ($C_{\text{AIR}}^{\text{MEASURED}}$). For each fungal type, the estimated values were greater than (or about the same as) the measured ones. The estimated total airborne spore concentration ranged from (31 ± 12) to $(50 \pm 7) \times 10^4/\text{m}^3$, while the actual concentration was between (1.4 ± 0.2) and $(17.1 \pm 4.3) \times 10^4/\text{m}^3$, confirming that the FSSST data may be used for the assessment of maximum potential exposure. In relative sense, the above fungal aerosol concentrations (both the estimated and measured in homes) are rather high. For example, a review article by Rao et al. (1996) referred to $C_{\text{AIR}}^{\text{MEASURED}}$ of up to approximately $10^4/\text{m}^3$ for typical water-damaged buildings (this level can be considered to represent extremely contaminated indoor environments).

Conclusions

A recently-developed FSSST, which allows assessing the potential of fungal sources to aerosolize spores into indoor air, was used to investigate the spore release from four building materials in mold-problem homes. The spores of different species were efficiently aerosolized by the FSSST from these materials, exhibiting the total spore release rate ranged approximately from 10^2 to $10^3/\text{cm}^2/\text{min}$. The data show that only a small fraction (not exceeding several percent) of the spores present on the surface are aerosolized by the FSSST although it creates very favorable aerosolization conditions. The relative efficiency of the total spore release was highest for concrete, followed by painted drywall, particleboard and wooden joist. The spore release efficiency was found to depend on the fungal type and growth conditions. The aerosol spore concentration estimated from the release rate data was found in most

of the cases to be greater than that actually measured in these environments with air sampling. The results suggest that the FSSST, which provides the most favorable conditions for the spore aerosolization, can be used for assessing the maximum potential exposure to airborne spores released from identified sources in homes.

Acknowledgements

This study was supported by the U.S. Department of Housing and Urban Development (Healthy Homes

Research, Grant OHLHH0099-01). Mr Niemeier was partially supported through NIOSH ERC Grant No. T42/CCT510420. This support is deeply appreciated. The authors are indebted to Mr Dainius Martuzevicius and Dr Mikhail Yermakov for their assistance in collecting field samples and to Ms Alexandra Appatova for her help in editing the manuscript.

References

- Ayerst, G. (1966) Influence of physical factors on the deterioration by moulds, *Soc. Chem. Ind. Monogr.*, **23**, 14–20.
- Becker, R. (1984) Condensation and mould growth in dwellings – parametric and field study, *Build. Environ.*, **19**, 243–250.
- Beguín, H. and Noland, N. (1994) Mould biodiversity in homes I. Air and surface analysis of 130 dwellings, *Aerobiologia*, **10**, 157–166.
- Brunekreef, B., Dockery, D.W., Speizer, F.E., Ware, J.H., Spengler, J.D. and Ferris, B.G. (1989) Home dampness and respiratory morbidity in children, *Am. Rev. Respir. Dis.*, **140**, 1363–1367.
- Burge, H.A. (1995) Aerobiology of indoor environment, *Occup. Med.*, **10**, 27–40.
- Burnett, J.H. (1976) *Fundamentals of Mycology*, London, Edward Arnold.
- Chang, J.C.S., Foarde, K.K. and Vanosdel, D.W. (1995) Growth evaluation of fungi (*Penicillium* spp. and *Aspergillus* spp.) on ceiling tiles, *Atmos. Environ.*, **29**, 2331–2337.
- Chang, J.C.S., Foarde, K.K. and Vanosdel, D.W. (1996) Assessment of fungal (*Penicillium chrysogenum*) growth on three HVAC duct materials, *Environ. Int.*, **22**, 425–431.
- Chew, G.L., Rogers, C., Burge, H.A., Muihlenberg, M.L. and Gold, D.R. (2003) Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics, *Allergy*, **58**, 13–20.
- Dales, R.E., Burnett, R. and Zwanenburg, H. (1991a) Adverse health effects among adults exposed to home dampness and molds, *Am. Rev. Respir. Dis.*, **143**, 505–509.
- Dales, R.E., Zwanenburg, H., Burnett, R. and Franklin, C.A. (1991b) Respiratory health effects of home dampness and molds among Canadian children, *Am. J. Epidemiol.*, **134**, 196–203.
- DeKoster, J.A. and Thorne, P.S. (1995) Bioaerosol concentrations in noncompliant, complaint and intervention homes in Midwest, *Am. Ind. Hyg. Assoc. J.*, **55**, 579–580.
- Duchaine, C. and Mériaux, A. (2001) The importance of combining air sampling and surface analysis when studying problematic houses for mold biodiversity determination, *Aerobiologia*, **17**, 121–125.
- Flannigan, B. and Morey, P.R. (1996) *Control of Moisture Problems Affecting Biological Indoor Air Quality*. ISIAQ guidelines: ISIAQ, Inc. Press, 1–67.
- Flappan, S.M., Portnoy, J., Jones, P. and Barnes, C. (1999) Infant pulmonary hemorrhage in a suburban home with water damage and mold (*Stachybotrys atra*), *Environ. Health Perspect.*, **107**, 927–930.
- Foarde, K.K., Vanosdel, D.W., Menetrez, M.Y. and Chang, J.C.S. (1999) Investigating the influence of relative humidity, air velocity, and amplification on the emission rates of fungal spores. In: Raw, G., Aizlewood, C. and Warren, P. (eds) *Proceedings of Indoor Air 99*, Vol. 1, London, CRC, 507–512.
- Galloway, L.D. (1935) The moisture requirement of molds fungi with special reference to mildew in textiles, *J. Text. Inst.*, **26**, 123–129.
- Górny, R.L., Reponen, T., Grinshpun, S.A. and Willeke, K. (2001) Source strength of fungal spore aerosolization from moldy building material, *Atmos. Environ.*, **35**, 4853–4862.
- Grant, C., Hunter, C.A., Flannigan, B. and Bravery, A.F. (1989) The moisture requirements of moulds isolated from domestic dwellings, *Int. Biodeterior.*, **25**, 259–284.
- Gravesen, S., Nielsen, P.A., Iversen, R. and Nielsen, K.F. (1999) Microfungal contamination of damp buildings-examples of risk constructions and risk materials, *Environ. Health Perspect.*, **107**, 505–508.
- Gregory, P.H. (1973) *The Microbiology of Atmosphere*, Plymouth, MA, Leonard Hill Books, 39–42.
- Grinshpun, S.A., Górny, R.L., Reponen, T., Willeke, K., Trakumas, S., Hall, P. and Dietrich, D.F. (2002) New method for assessment of potential spore aerosolization from contaminated surfaces. *Proceedings of the Sixth International Aerosol Conference*, Vol. 2, Taipei, Taiwan, 767–768.
- Hukka, A. and Viitanen, H.A. (1999) A mathematical model of mould growth on wooden materials, *Wood Sci. Technol.*, **33**, 475–485.
- Hyvärinen, A., Reponen, T., Husman, T., Ruuskanen, J. and Nevalainen, A. (1993) Characterizing mold problem buildings – concentrations and flora of viable fungi, *Indoor Air*, **3**, 337–343.
- Icenhour, C.R. and Levetin, E. (1997) *Penicillium* and *Aspergillus* species in the habitats of allergy patients in Tulsa, Oklahoma area, *Aerobiologia*, **13**, 161–166.
- Kildeso, J., Würtz, K.F., Nielsen, P., Kruse, K., Wilkins, K., Tharne, U., Gravesen, S., Nielsen, P.A. and Schneider, T. (2003) Determination of fungal spore release from wet building materials, *Indoor Air*, **13**, 148–155.
- Lacey, J. and Crook, B. (1998) Fungal and actinomycete spores as pollutants of the workplace and occupational allergens, *Am. Occup. Hyg.*, **32**, 515–533.
- Macher, J.M. and Huang, F.-Y. (1991) A two year study of microbiological indoor air quality in a new apartment, *Arch. Environ. Health*, **46**, 25–29.
- Madelin, T.M. (1994) Fungal aerosol: a review, *J. Aerosol Sci.*, **25**, 1405–1412.
- Meyer, H.W., Würtz, H., Suadicani, P., Valbjørn, O., Sigsgaard, T., Gyntelberg, F. and Members of a working group under the Danish mould in buildings program (DAMIB) (2004) Molds in floor dust and buildings-related symptoms in adolescent school children, *Indoor Air*, **14**, 65–72.
- Nielsen, K.F. (2003) Mycotoxin production by indoor molds, *Fungal. Genet. Biol.*, **39**, 103–117.
- Nielsen, K.F., Nielsen, P.A. and Holm, G. (2000) Growth of moulds on building

- materials under different humidities. In: Seppänen, O. and Säteri, J. (eds) *Proceedings of Healthy Buildings 2000*, August 6–10, 2000, Espoo, Finland, SYI Indoor Air Information Oy, Helsinki, 283–288.
- Pasanen, A.-L., Pasanen, P., Jantunen, M.J. and Kalinoski, H.T. (1991) Significance of air humidity and air velocity for fungal spore release into the air, *Atmos. Environ.*, **25A**, 459–462.
- Pasanen, A.-L., Niinen, M., Kalliokoski, P., Nevalainen, A. and Jantunen, M.J. (1992) Airborne *Cladosporium* and other fungi in damp vs. reference residences, *Atmos. Environ.*, **26B**, 117–120.
- Pasanen, A.-L., Kasanen, J.-P., Rautiala, S., Ikäheimo, M., Rantamäki, J., Kääriäinen, H. and Kalliokoski, P. (2000) Fungal growth and survival in building materials under fluctuating moisture and temperature conditions, *Int. Biodet. Biodeg.*, **46**, 117–127.
- Peat, J.K., Dickerson, J. and Li, J. (1998) Effects of damp and mould in the home on respiratory health: a review of the literature, *Allergy*, **53**, 120–128.
- Pirhonen, I., Nevalainen, A., Husman, T. and Pekkanen, J. (1996) Home dampness, moulds and their influence on respiratory infections and symptoms in adults in Finland, *Eur. Respir. J.*, **9**, 2618–2622.
- Rand, T. (1999) An assessment of mould contamination problems in Atlantic Canada schools: mold burdens, amplifying sites and benefits of proactive school inspection policies. In: Johanning, E. (ed.) *Bioaerosols, Fungi and Mycotoxins: Health Effects, Assessment, Prevention and Control*, Albany, Eastern New York Occupational and Environmental Health Center, 581–592.
- Rao, C.Y., Burge, H.A. and Chang, I.M. (1996) Review of quantitative standards and guidelines for fungi in indoor air, *J. Air Waste Manage. Assoc.*, **46**, 899–908.
- Rautialai, S., Reponen, T., Hyvärinen, A., Nevalainen, A., Husman, T., Vehviläinen, A. and Kalliokoski, P. (1996) Exposure to airborne microbes during the repair of moldy buildings, *Am. Ind. Hyg. Assoc. J.*, **57**, 279–284.
- Rowan, N.J., Johnstone, C.M., McLean, R.C., Andersen, J.G. and Clarke, J.A. (1999) Prediction of toxigenic fungal growth in buildings by using novel modeling system, *Appl. Environ. Microbiol.*, **65**, 4814–4821.
- Scott, W.J. (1957) Water relations of food spoilage micro-organisms, *Adv. Food Res.*, **7**, 83–127.
- Sivasubramani, S.K., Adhikari, A., Reponen, T., Willeke, K. and Grinshpun, S. (2002) Source tester for fungi aerosolized in indoor environments: development and evaluation of the new concept. *Proceedings of the Indoor Air Quality-Filtration Conference*, Cincinnati, Ohio, November 14–15.
- Sivasubramani, S.K., Niemeier, R.T., Reponen, T. and Grinshpun, S.A. (2004) Fungal spore source strength tester: laboratory evaluation of a new concept, *Sci. Total Environ.* (in press).
- Spengler, J., Neas, L., Nakai, S., Dockery, D., Speizer, F., Ware, J. and Raizenne, M. (1993) Respiratory symptoms and housing characteristics, *Proc. Indoor Air*, **1**, 165–168.
- Verhoeff, A.P. and Burge, H.A. (1997) Health risk assessment of fungi in home environments, *Ann. Allergy Asth. Immunol.*, **78**, 120–128.
- Verhoeff, A.P., van Wijnen, J.H., Boleij, J.S.M., Brunekreef, B., van Reenen-Hoekstram, E.S. and Samson, R.A. (1990) Enumeration and identification of airborne viable mould propagules in houses, *Allergy*, **45**, 275–284.
- Viitanen, H.A. and Bjurman, J. (1995) Mould growth on wood at fluctuating humidity conditions, *Mater. Organismen.*, **29**, 27–46.
- Wickman, M., Gravesen, S., Nordvall, S.L., Pershagen, G. and Sundell, J. (1992) Indoor viable dust-bound microfungi in relation to residential characteristics, living habits, and symptoms in atopic and control children, *J. Allergy Clin. Immunol.*, **89**, 752–759.
- Zoberi, M.H. (1961) Take-off mold spores in relation to wind speed and humidity, *Ann. Bot.*, **25**, 53–64.