Development of Experimental Procedure to Evaluate Potential Movement of Mold Spores from Wall Cavity to Indoor Environment

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ABSTRACT

Molds and decay in building envelopes have recently gained the attention of building envelope researchers. Among various mold related issues, information is needed on the capacity of building envelopes to contain small amounts of molds, from either slightly moldy materials used in construction or minor growth of molds during occupancy. A testing program is carried out on full-scale wood framed envelope systems to monitor the transport of mold spores from the stud cavity to the indoor environment. In the test setup, the wall specimen (3' × 8' high) is enclosed on the interior by a sampling chamber and on the exterior by a filtering chamber. Both chambers are made up of a shallow 5-sided metal box. During the test a mold source, consisting of either visible surface molds from wood studs or of spore surrogate particles (polystyrene microbeads), is placed within the stud cavity; a negative pressure in the sampling chamber is established to create an air infiltration through the wall specimen. Air samples are taken for the spore amounts in the sampling chamber and stud cavity, as well as in the filtering chamber and laboratory for establishing background levels. A total of 16 tests are performed with variations of envelope parameters in characteristics of air leakage and presence or absence of insulation and vapor barrier. In addition to spores, other fungal components and products due to molds and mold growth on building materials within the insulation cavity are also monitored. In this paper, the test setup and two trial runs on spore movements are presented.

1. INTRODUCTION

Moisture damage to building envelopes has been present in many envelope failure cases in North America due to excessive rain penetration, such as the well-known cases of Vancouver condos, North Carolina EIFS housing units, and Seattle leaky homes. In most of these cases, mold growth and decay were present. Mold is present in all indoor conditions, for example, the molds on construction materials and minor growth of molds within the envelope; however, damp indoor conditions increase significantly the likelihood of problems for occupants. Field investigations (e.g. Lawton et. al., 1998) indicate that moldy houses can have significantly higher spore concentration and other microbiological contamination.

The building envelope serves as a filter between the indoor and outdoor environments to provide a comfortable and healthy indoor living space. One of the important functions of the envelope is to limit access of organisms (including fungi) to the interior space. A research project has been undertaken to establish to what extent building envelopes of different wall configurations can contain molds that may exist within their stud cavities. A laboratory experiment program has been initiated to investigate whether mold propagules, such as spores and microbial volatile organic compounds, MVOCs, generated from within the envelope can "penetrate" through the wall and enter the indoor living space by air infiltration. This paper presents the concept and design for the project. Results from two initial tests on mold spore

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sampling are discussed. While the project includes sampling and analysis of fungi biomass indicators other than spores (such as MVOC, ergosterol and glucan) and of spore surrogates (microbeads), this paper is limited to issues related to fungal spores.

2. LITERATURE REVIEW

Fungus is one of the oldest life forms on the earth and makes up approximately 25% of the earth's biomass. Fungi can reproduce either sexually or asexually through the production of large numbers of spores. Among difference transport means, airborne spores can travel with the air and reach other surface of potential food sources. When the conditions are suitable, viable spores germinate to produce hyphae, which grow and spread within or on the substrate, and eventually form a new generation of fungi.

Molds are filamentous fungi and have a powdery look due to the millions of spores produced by the fungi. Molds are found in buildings and walls in moisture-damaged homes. Exposure to indoor molds could pose health risks and cause adverse health effects to the occupants (Miller, et al. 1997). Linkages between health effects and moisture and mold problems of buildings have been described in the literature, even though the exact mechanisms and the correlation between the causative agents and the symptoms remain poorly understood (Bornehag et al., 2001) (NAS, 2004). The adverse health effects include allergenic reactions, infection, and toxic responses (Górny et al., 2002). Many studies have documented an increase in the risk for certain symptoms among occupants of moisture and mold damaged buildings (Bornehag, et al. 2001; Haverinen, 2001 & 2003). The health effects depend on factors such as the species involved, the fungal products produced, the severity and duration of the contaminated environment that occupants are exposed to, and different susceptibility of the occupants.

Transport of spores by air movement has long been studied in the fields of mycology and aerobiology. The mechanisms and processes for their movement through building envelopes are even more complex and involve release and dispersion, deposition and re-suspension, agglomeration, and other aerodynamic processes (Reponen, 1995). Many factors can influence airborne fungal spore release and release rates: the materials, texture of the surface, the fungal species, relative humidity, temperature and their variations, air velocity over the surface, and mechanical vibration of the structure (Kildesø et al., 2002). Gorny et al (2001) determined that release of spores into air currents was greater from building materials with rough surfaces due to increased turbulence at the surface of the material.

Backman et al. (2000) traced the movement of two fungal species through expansion joints and pipe channels into the occupied space in school rooms under negative pressure due to the mechanical ventilation system. Matilainen and Pasanen (2002) analyzed airborne fungal spores from indoor, outdoor and crawlspace in eight buildings and found that microbes were being transported from crawlspaces into the living space through air leakage in the floor. However, they did not quantify or relate the spore counts and specific leakage paths. Suonketo and Pessi (2000) measured air-transported microbes from the insulation inside exterior precast concrete walls in a field study of nine apartments under renovation. In their experiments, a chamber was attached from the indoor side to the wall sections with visible cracks on the inner surface. The chamber was depressurized to induce air leakage through the covered section. Air in the chamber was sampled. The results indicated that large air leakage could transport microbes within the walls into the living space.

Airaksinen et al. (2004) carried out laboratory experiments on inert particles and spores penetrating through floors that that separated the crawl foundation and living space in typical Finnish residential buildings. A full-sized wood framed floor structure was built in the laboratory, and placed between two chambers; one corresponds to indoor space and the other to the crawlspace. The penetration of spores and inert particles was measured when structure, pressure difference and air leakage rates were varied. They found that the penetration of spores was highly dependent on the pressure difference rather than holes or cracks on the surface. The result indicated that it is difficult to prevent spores through floors by sealing.

In a related field on airborne particulate matters, there are much more established and extensive researches in filtration theories and experiment work. Among them, many studies were related to particle penetration

through walls. For example, Liu and Nazaroff (2003) used filtration theory to calculate penetration fractions for particles (and other pollutants) in air infiltration through various air leakage paths and envelope materials. Mosley et al. (2001) performed lab experiments to study the mechanisms and penetration factors of particles through slot-shaped cracks in various envelope materials. Taylor et al. (1999), in their study of dynamic walls that utilize intentional airflow within the wall to improve their thermal performance, considered the building envelope as particle filters that capture particles in air through interception, impaction and deposition. Tung et al. (1999) carried out field studies in office buildings on particulate penetration coefficients through the envelope including cracks of door frames. Chao et al. (2003) estimated particle penetration coefficients of envelopes from field measurements in apartment residences.

More robust and extended studies on the spore transportation through building envelopes have yet to be carried out. One application of such research is to evaluate the effectiveness of envelope wall systems in preventing movement of spores into the living space, especially for the spores that may exist in the wall due to the use of lumber that may have typical levels of mold as expected on some construction sites.

3. EXPERIMENTAL DESIGN

The on-going project investigates whether the envelope can prevent minor mold growth within the stud cavity from reaching the living space. The influence of envelope components on the containment are investigated through a laboratory test setup on full-size wood framed wall assemblies. The Test A hypothesis is to differentiate between levels of spore and VOC measurement adjacent to a stud space containing contaminated wood, compared with a stud space containing clean wood.

For a wall specimen, one full stud space is taken as being representative of the walls in wood-framed residential buildings. The specimens are built with 38mm x 140mm (2"x6" nominal size) wood studs and flanked by two guarded bays of eight inches each, as shown in Figure 1. A boundary made up of plywood boards contains the entire specimen to service as supports for sampling and depressurization apparatus. The specimen layers include cladding, sheathing, and interior drywall and finish, as shown in Figure 2.

There are many envelope and mycological factors that could influence the movement of spores from within the insulation cavity through the drywall. Sixteen (16) testing scenarios, as shown in Table 1, are designed to investigate the following parameters:

- presence of mold sources by using "moldy" studs or clear studs,
- two patterns of air leakage paths through the wall section,
- insulated or non-insulated cavity, and
- presence or absence of vapor barrier.

Two special metal chambers are constructed to encase the central insulation cavity of the specimens, as shown in Figure 1. One chamber seals off the outdoor side of the specimen and allows the entry of conditioned air with designed relative humidity levels; the other on the indoor side maintains specified pressure differentials or air leakage rates across the specimen.

Air leakage rates of a specimen vary up to 12 or 25 LPM. The values correspond to the average wall leakage rates of a house with assumed air exchange rates of 0.75 and 1.5 ACH (air exchange per hour), and with the assumption that 1/3 air leakage occurs through walls uniformly. Two patterns of air leakage are used. The *long-path* allows air to enter at a lower electrical box and leave at the top header, while the direct path allows air to enter and leave at the electrical box level. Air leakage paths are provided by holes on the panels with variable cross-sections. The air leakage is induced by a suction pump connected to an opening port on the sampling chamber. Airflow rates are regulated by a manually operated needle valve and measured by an inline flow meter (laminar flow element).

FIGURE 1. SKETCH OF TEST SETUP

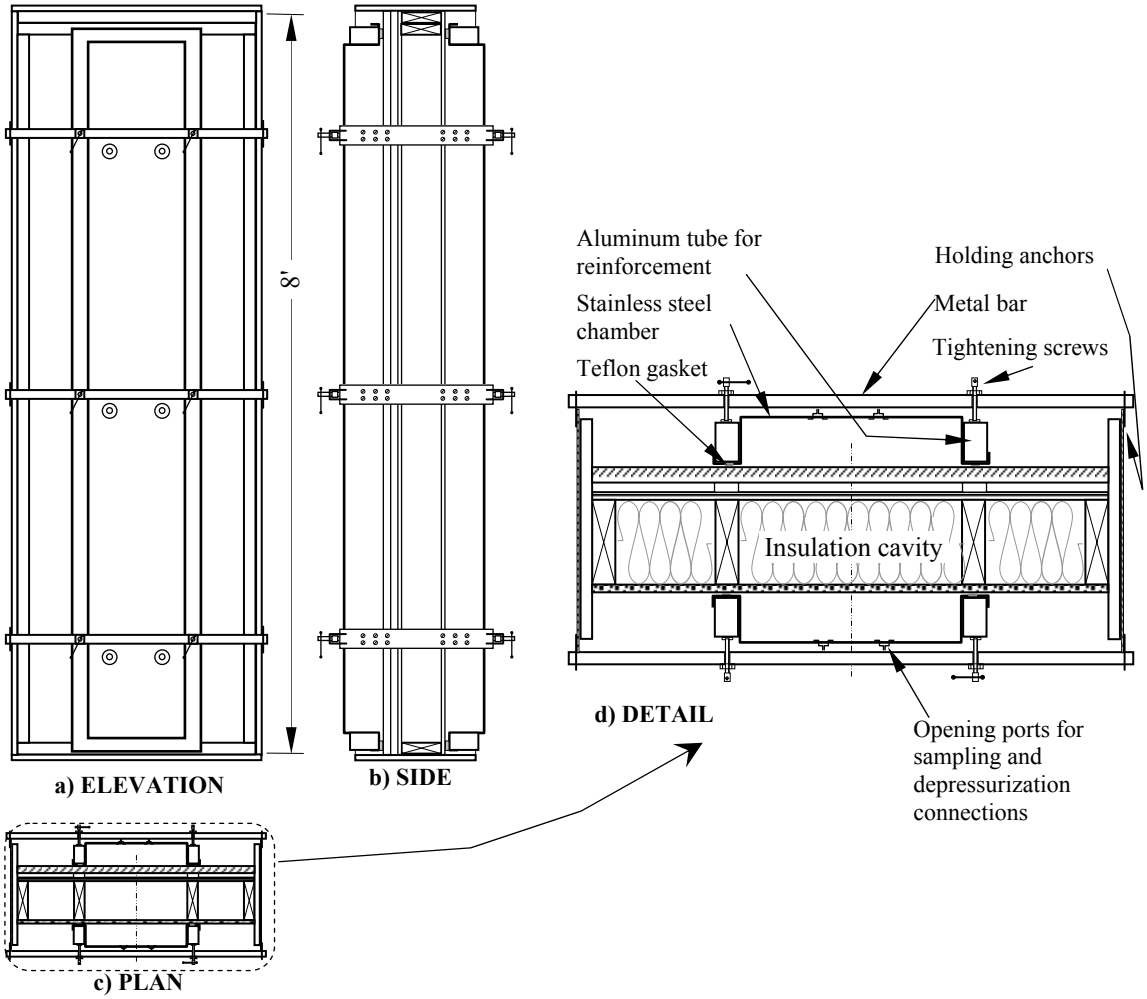
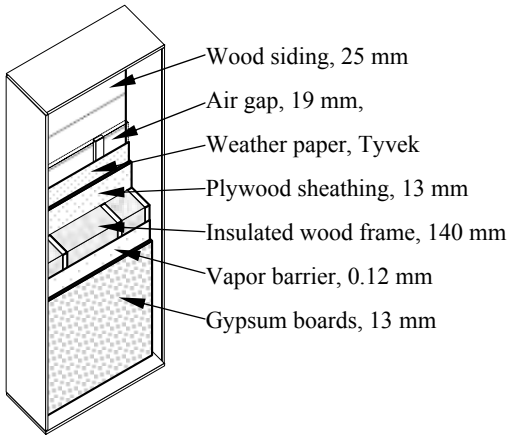


FIGURE 2. A PHOTO AND ISOMETRIC VIEW OF THE SPECIMEN



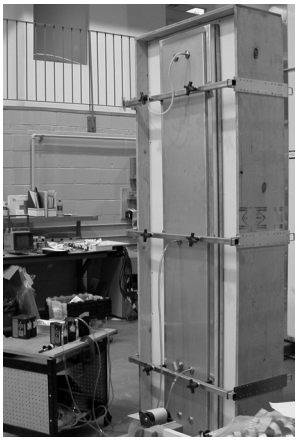


TABLE 1. SPECIMEN CONFIGURATION FOR MOLD TEST A

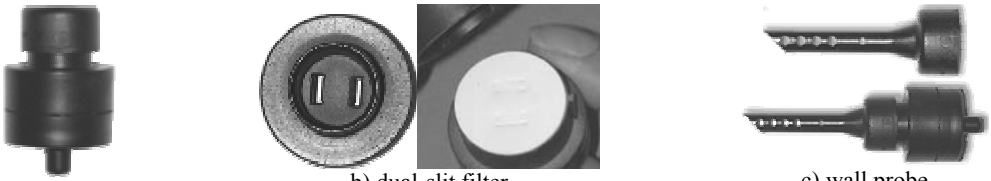
#	Spore Source	Air Leakage		cavity insulation	vapor barrier
		Rate	Path		
1	no	no	long path	yes	yes
2	moldy studs	no		no	no
3		low		no	no
4		high		no	no
5		low		yes	no
6		high		yes	No
7		low		yes	yes
8		high		yes	yes

#	Spore Source	Air Leakage		cavity insulation	vapor barrier
		Rate	path		
9	no	no	direct path	yes	yes
10	moldy studs	no		no	no
11		low		no	no
12		high		no	no
13		low		yes	no
14		high		yes	no
15		low		yes	yes
16		high		yes	yes

The process for preparing the moldy studs in the laboratory setting contains several steps. First, the studs are fully immersed into water for more than 7 days or till the moisture contents of the studs reach about 40% (by weight). Second, studs are placed inside an airtight box to grow molds on the surfaces. Metal trays containing garden soils and trays with water are placed under the raised studs in the box. Third, the extent of mold growth is checked periodically, confirmed by mold sampling and expert opinion, until 10% visible mold coverage is reached. Finally, the studs are dried with forced air convection to 16% moisture contents and are ready for use.

Special cassettes (Bi-Air cassette from Bi-Air Corp.) were selected for the spore sampling. The cassette is designed for both total spore counts and culturing on the same sample. A dual-slit filter is used to produce two distinct sample traces of 9.35 mm^2 each. The filter is cut in half to allow each sample trace to be analyzed for total fungal spores counted microscopically and for viable fungi using dilution plating techniques. The cassette can be used together with a perforated sample probe for sampling through drywalls. Figure 3 shows images of the cassette and wall probe. After spore sampling, the cassettes are capped from both ends and transported to a mycology lab for processing and analysis. In the laboratory, the total fungal spores are counted under a microscope by using one sample trace on the dual-slit filter, and viable fungi are cultured on 2% malt extract agar by using the other trace.

FIGURE 3. CASSETTE AND WALL PROBE



a) cassette b) dual-slit filter c) wall probe

4. TRIAL TESTS AND RESULTS

Two trial runs were carried out to evaluate the test setup and sampling equipment and procedures for mold spores. The setup used is shown in Figure 1. The specimen had neither insulation nor vapor barrier. Moldy bread bits were placed at the bottom of the insulation cavity to serve as the only mold (spore) source. Materials used to assemble the specimen had no visible molds. The molds were grown by placing bread bits on top of soil trays, which were sealed in a large plastic container with 1/2" of water at the bottom. The bread in the tray was left for 7 days and heavy mold growth was detected before use. Air

leakage paths were provided through three purposely-drilled ϕ1/2" holes on both sheathing and drywall at 12" from the bottom plate. The air leakage rate was set at 11.0 LPM (liter per minute) during sampling, with 1.0 LPM through the sampling tube and 10.0 LPM by the depressurization setup.

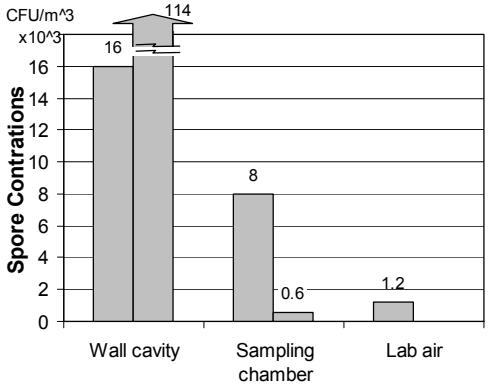
Before the trial runs, the "bread tray" was placed in the specimen and the drywall was installed. The stainless steel sampling chamber was tightened to the drywall, with W-shaped rubber gaskets in between for airtightness. The chamber on the sheathing side was not used. For the insulation cavity, a wall sampling probe and cassette were directly inserted into one hole through the drywall. For the sampling chamber, the sampling cassette was placed inline with the tube connected to the sampling port. The depressurization tube was connected to another sampling port. The flow rates through the filters sampling tube (1.0 LPM) were calibrated with a flow calibrator (DC-Lite Flowmeter by SKC Inc).

Results from five samples taken during the first run are shown in Table 2 and Figure 4. Two samples were taken from the wall cavity before the sampling chamber was attached, two from the sampling chamber during depressurization, and one taken directly from the lab air.

TABLE 2. SPORE CONCENTRATION FROM RUN #1

#	Location	Duration	CFU	CFU/m^3 ($\times 10^3$)
1	Wall cavity	10 min	80	16.0
2		20 min	1140	114.0
3	Sampling chamber	20 min	6	0.6
4		20 min	80	8.0
5	Lab air	10 min	6	1.2

FIGURE 4. SPORE CONCENTRATIONS MEASURED IN 1st RUN



In the laboratory, the cassettes were opened and each filter was cut in half. One half of the filter was placed in a sterile plastic tube containing 10 ml of sterile distilled water. The tube was agitated vigorously for 5 min to release all spores from filter to water solution. An amount of 0.5 ml of spore solution was evenly applied to a Petri plate holding 20 ml of a 2% malt extract agar medium. Three Petri plates were inoculated with one spore solution. The plates were placed at 25ºC for 3 days, and colony forming units (CFU) in each plate were counted. If the CFU in the plate was too high for counting, the spore solution was further diluted to 100 and 500 times with sterile distilled water, and the process was repeated with diluted solutions. CFU for each sample was calculated based on the average of three replicate plates. The other half of the filter was immediately placed on a glass slide loaded with a drop of Triacelin. The slide was gently heated over an alcohol lamp until the filter became clear. The filter was then covered with a cover slip. The slide was observed under a light microscope at a magnification of 500X, and the number of spores that

appeared on the filter was counted. For results from culturing, the spore concentration was calculated according to:

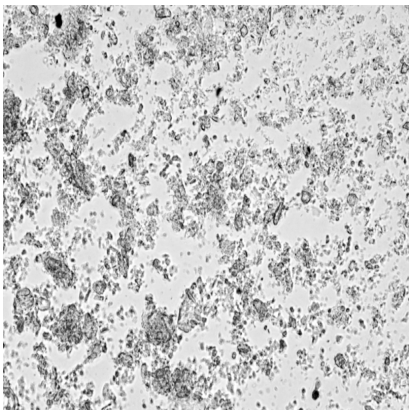
$$\text{Spore concentration (CFU/m}^3\text{)} = \frac{2 \times 1000 \times \text{CFU}_{\text{cultured}}}{\text{sampling flow rate (LPM)} \times \text{sampling time (min)}}$$

where the double factor, "2", in the equation is because only one of the two sample traces is used for culturing.

The result indicated, as expected, that spores had high concentrations at the source-the empty insulation cavity, and much fewer spores were found in the sampling chamber, while the background spore concentration in the lab space was much lower than both. Two extreme values were observed within the data. One relatively high concentration was measured in the stud cavity (114×10^3 CFU/m^3). This value was obtained when the wall specimen was hammered with a mallet to induce vibrations on the sample. This procedure will not be used in the tests. The other very low value (0.6×10^3 CFU/m^3) was obtained in the sampling chamber.

From the sampling analysis, the first trial run suggested improvements in conditions and duration of the sampling. Under a microscope, too many dust particles were found on the filter membrane, as illustrated in Figure 5 by a photo of the microscope image of the sample #1 (in the wall cavity). It was very difficult even for personnel with high technical training and experience to distinguish any fungal spores among these particles. Therefore, no counting results were included for this test run in Table 2. The dust-on-filter issue indicates that the 10-20 min sampling time in a wall cavity was too long and the vibrations induced on the test specimen by hammering should not be used in later tests.

FIGURE 5. MICROSCOPIC IMAGE OF SAMPLE #2 OF TRIAL RUN #1



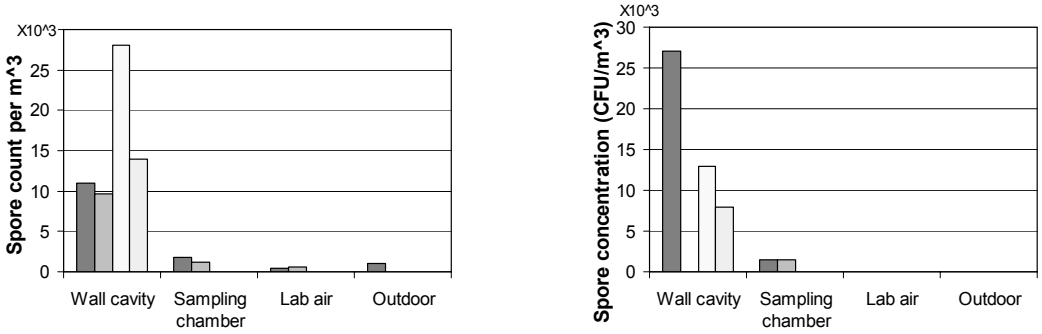
A second trial run was conducted with shorter sampling durations. Sampling of the wall cavity was repeated with 2 and 5 min durations, while the times for the sampling chamber were reduced from 20 min to 10 min. In addition, the specimen was let alone without any purposely-induced vibration after moldy bread was inserted and the wall and sampling chamber tightened. The test setup and procedure were essentially the same as the previous run. The analysis results were shown in Table 3 and Figure 6. With the reduced sampling time and no vibrations, there were much fewer dust particles on the filter membrane and microscopic counting provided good results. Figure 7 shows an enlarged image of one sample.

The spore counts from microscopy counting and concentration from culturing for run #2 show compatible values. This indicates that the collected spores came from the moldy breads and most of the spores are newly released and viable.

TABLE 3. LABORATORY ANALYSIS OF AIR SAMPLES TAKEN IN TRIAL RUN 2

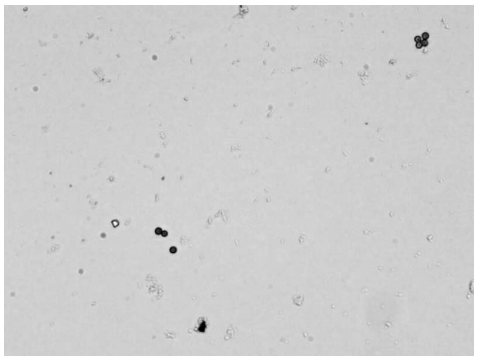
#	Location	Time	Microscopy count ($\times 10^3$)	CFU/m^3 ($\times 10^3$)
1	Wall cavity	2 min	11	27
2		5 min	9.6	0
3		2 min	28	13
4		5 min	14	8
5		5 min	1.2	0
6	Sampling chamber	10 min	1.8	1.4
7		10 min	1.2	1.4
8	Lab air	20 min	0.4	0
9		10 min	0.6	0
10	Outdoor	20 min	1.1	0

FIGURE 6. SPORE CONCENTRATIONS FOR THE SECOND TRIAL RUN



a) microscope count b) culturing

FIGURE 7. MICROSCOPIC IMAGE OF SAMPLE #1 IN RUN 2



5. DISCUSSION

The trial runs indicate that the proposed method of testing is feasible. All three data sets of related spore amounts inside the wall and sampling chamber showed that the concentrations/counts in the sampling chamber is lower than those in the stud cavity. After taking out extreme values and averaging available

data in each data set, the ratio between the amount in the sampling chamber to that of the wall cavity can be calculated, as listed in Table 4. The value for the trial run 1 is 0.5 and 0.13 for both the counting set and culturing set in run 2.

TABLE 4. PENETRATION RATIOS

Data sets			Penetration ratio	Notes
Description	**wall cavity**	**chamber**		
A. Culturing, Run 1, Table 2, Figure 4	16,000	8,000	0.5	Extreme values (57,000 and 300) are removed
B. Counting, Run 2, Table 3, Figure 5a,	11,000; 9,600; 14,000	1,800; 1,200	0.13	The largest value, 28000, is omitted
C. Counting, Run 2, Table 3, Figure 5a,	13,000; 8,000	1,400; 1,400	0.13	Value, 27000, is omitted

The relatively higher ratio for Run #1 may be because that the vibrations were introduced during Run #1 while Run #2 was performed without vibration. However, without additional results from more test runs, the values obtained in these two test runs cannot be used for drawing any further general conclusions.

A potential issue related to the penetration ratio is the mixing of air inside the sampling chamber. When the sampling chamber is attached, there are no spores (except for the laboratory background concentration) in it. As air is infiltrated into the chamber and extracted by the depressurization setup and the sampling pump, more and more spores enter the sampling chamber. Thus the spore concentration there increases until it approaches a certain level. This level depends on the penetration ratio and could be a constant value if the spore concentration in the cavity is constant and spores are inexhaustible for the period of testing. Therefore, the spore concentration in the sampling chamber, and thus, the derived penetration ratio, can be affected by when the sampling is taken. A simple solution is to let air flow through the specimen and sampling chamber before sampling is performed. The duration of this airflow is affected by the ratio between the air infiltration rate, the volume of the sampling chamber and how well the air that infiltrates through the drywall mixes with the air inside the sampling chamber; as well as more complex aerobiological mechanisms of surface settling and resuspension of particulates from the inner surface covered by the sampling chamber. This duration is set to a value long enough for twice the volume of air in the sampling chamber to be extracted. With the assumptions of perfect mixing in the sampling chamber and constant spore concentration in the wall cavity, this duration allows the concentration in the sampling chamber to reach about 87% of the final level.

Another factor to influence spore movement is the fiberglass insulation. Existing research (such as Liu and Nazaroff, 2003) on fine airborne particulate matter (diameters 0.02 to 10 μm) concluded that glass fiber insulation used in wall cavities is very efficient in filtering out particles in infiltrating air. Assuming there are no air gaps in the insulation, nearly all particles are filtered by the fibers in several inches of air path through the insulation. Therefore, in later tests fiber batt insulation is expected to capture all particles before they reach any air cracks on the drywall. While mold spores may have a different aerodynamic behavior, the detection of any significant numbers of spores, as in the trial runs without insulation presented above, may still be unlikely for the sampling chamber, especially considering that the mold spore source on the vertical studs is not generally on the direct air leakage path.

The actual runs for the planned tests also have vapor barrier behind the drywall in addition to insulation. It is expected that the penetration ratios are different, most probably much smaller. Spore concentrations in the sampling chamber, therefore, are likely to be much lower than those observed in these preliminary tests. The sampling parameters, airflow rates and durations, need modifications to account for the changes.

ACKNOWLEDGEMENTS

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