

Lateral fungal spore movement inside a simulated wall

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Received: 27 June 2008 / Accepted: 6 October 2008
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Abstract Moisture inside walls can facilitate mold growth if left untreated. Once spores become airborne they may interact with pressures inside walls. Two laboratory experiments were conducted to determine if airborne spores have the potential to migrate laterally inside walls with and without wiring installations. A simulated wall was fabricated, and *Penicillium chrysogenum* spores were aerosolized into a distant stud bay and an adjacent stud bay. The wall was subjected to a typical indoor pressure. Spore levels inside the bays were sampled, and a total of 36 trials ($n = 36$) were conducted. Results of Kruskal–Wallis tests revealed that spore levels inside the sampling bay and the distant bay with wiring installations were not significantly different. Spore levels inside the sampling bay were significantly lower than the adjacent bay without wiring installations ($P < 0.05$). The findings of the study suggest

airborne fungal spores have the potential to move laterally inside walls.

Keywords Airborne spores · Fungal spores · Indoor air quality · Interior wall · Mold

1 Introduction

Mold in the indoor environment has been associated with building-related illness (Finnegan et al. 1984). Wall cavities with chronic moisture problems due to leaky plumbing or natural flooding have the potential to act as artificial incubation chambers for molds (Heinsohn 2007). Once moisture becomes established in construction materials, sugars in sapwood joists and starch adhesives in gypsum wallboards can provide nutrient-rich surfaces for mold growth (Laks et al. 2002). Some commercially available gypsum wallboard also contains a ‘baseline bioburden’ of dormant mold that will colonize under high humidity conditions (Price and Ahearn 1999). Mold growth on wooden studs even has the potential to undermine the integrity of a building over time (Chung et al. 1999). Wall cavities are also subjected to pressure changes associated with air flows inside the building envelope and may be related to temperature changes, stack effects and mechanical ventilation. TenWolde et al. (1995) observed that 18 mile h^{-1} winds buffeting exterior walls induced pressure differences of up to

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20.0 Pa inside wall cavities. Mosley et al. (2001) consider pressure changes of up to 20.0 Pa to be common between indoor and outdoor spaces.

Several studies have examined artificial particle penetration through structural components. Liu and Nazaroff (2003) measured particle penetration through cracks in wall materials in a laboratory setting. Latex particles were passed through cracks in aluminum, brick, concrete, plywood, redwood, pine and sheathing wall materials. The results of the experiment indicated that complete penetration occurred for particles up to 7.0 μm in diameter when exposed to 4.0 Pa of pressure and when the crack height was 1.0 mm or greater. Mosley et al. (2001) examined penetration for particles 2.0 and 5.0 μm in diameter through 0.5-mm-high simulated cracks under pressures of 2.0, 5.0, 10.0, and 20.0 Pa, respectively. Their results showed that penetration for 2.0- μm -diameter particles increased substantially as pressures increased but that the penetration of 5.0- μm particles only increased substantially when pressures reached 20.0 Pa. Liu and Nazaroff (2002) simulated particle penetration through windows in a laboratory setting. The windows were positioned between two plywood chambers, and one side was depressurized to -1.0 Pa. The results of this experiment revealed that particles with diameters smaller than 0.4 μm and larger than 2.0 μm had a higher penetration through windows without weather stripping than through windows with weather stripping and that 80% of particles between 0.2 and 3.0 μm in diameter penetrated both windows.

Only a few studies have examined mold spore movement between building components. Buttner et al. (1999) demonstrated that HVAC (heating, ventilating, and air conditioning) systems in a laboratory setting have the potential to allow the movement of airborne spores into a simulated room. Airaksinen et al. (2003) found that spore movement was possible through residential flooring held vertically between two chambers inside a laboratory. Fazio et al. (2005) examined spore exfiltration through simulated walls to test a new penetration procedure. Several full-sized exterior wall cavities were constructed, the studs were inoculated with fungi, and the colonies were allowed to grow. Two chambers were positioned outside the walls of the cavities. During the trials, one chamber was depressurized, and air flowed from the cavity to the chamber

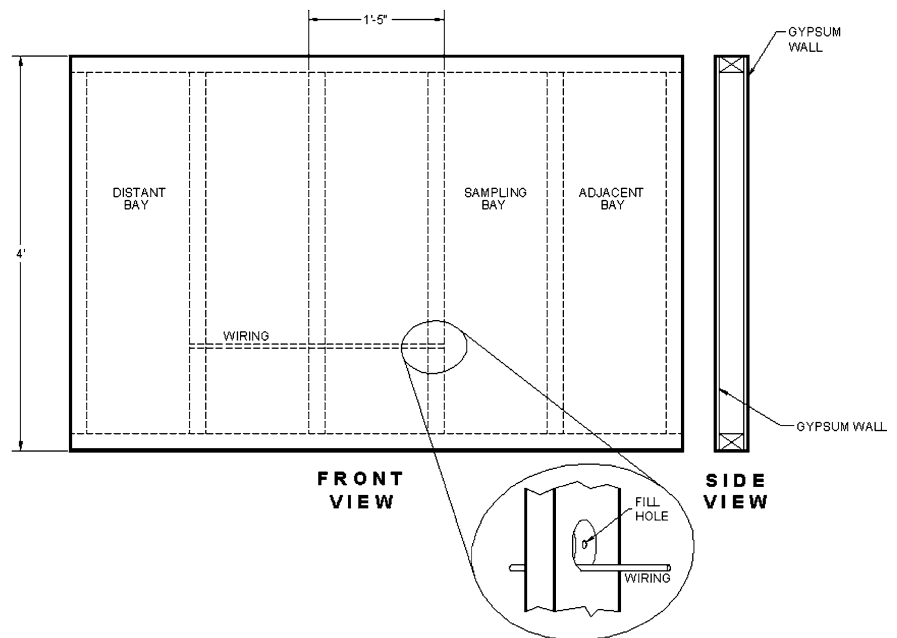
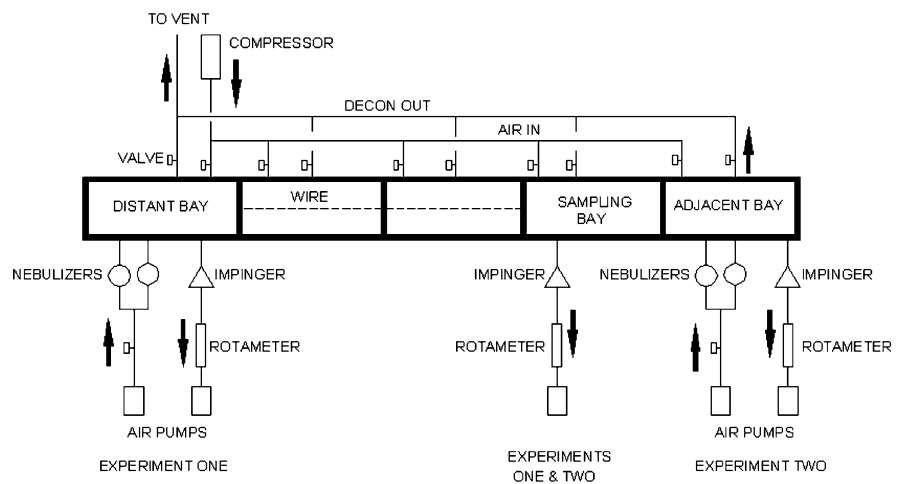
through holes in the plywood walls. The results of this experiment revealed that the spores had moved through the wall cavities and into the depressurized test chamber. Rao et al. (2006) statistically evaluated the chamber procedure and concluded that spore counts were significantly higher ($P = 0.02$) inside cavities with direct air flow paths than in cavities with longer air flow paths.

The objective of the study reported here was to determine whether or not airborne mold spores under 20.0 Pa of interstitial pressure have the potential to move laterally through multiple stud bays with wiring installations and to move through an adjacent stud bay under 20.0 Pa of interstitial pressure without wiring installations. In order to conduct these experiments, we fabricated a simulated wall with multiple stud bays.

2 Methodology

2.1 Materials

During the two experiments, an 8-foot long, 4-foot high residential internal wall with five stud bays was fabricated from 2×4 -inch wooden studs spaced 17-inch apart and covered with ToughRock gypsum wallboard (Georgia-Pacific, Atlanta, GA). The wall board was fastened to the studs with dry wall screws and sealed along the perimeter with carpenter's glue. All materials are commercially available. The wall's height simulated the lower half of an internal wall separated by fire blocks. Half-inch holes were drilled in three of the studs, and a conventional electrical two-wire with ground was installed between the studs to simulate a wall with utility lines. A small 1/4-inch horizontal fill hole was also drilled to intersect the wiring hole at the sampling stud from the rear of the wall to allow a bay to be sealed off from the outside with foam sealant. The small hole was plugged at the exterior when not needed. One bay was designated as the *sampling bay*. The bay farthest to the left with wiring installations was designated the *distant bay* and was three bays away from the sampling bay. The bay farthest to the right, with no wiring installations adjacent to the sampling bay, was designated the *adjacent bay*. Spore nebulizer, air pressure supply conduits, and decontamination egress conduits were installed on top of the three stud bays. Air sampling

Fig. 1 Wall setup**Fig. 2** Supply lines

conduits were also installed along the front of the three bays. The wall setup is illustrated in Fig. 1, and the supply lines are illustrated in Fig. 2.

2.2 Target fungi

Penicillium chrysogenum cultures (no. 15–6146) were purchased from Carolina Biological (Burlington, NC). The fungus is commonly found in approximately 68% of water-damaged buildings (Gravesen et al. 1997) and is considered to be a “primary colonizer” of construction materials with a low moisture content (Grant 1985). These spores

were well suited for this study because they are spherical with a diameter between approximately 1.0 and 3.0 μm , while most fungal spores have diameters between 2.0 and 10.0 μm (American Thoracic Society 1997). Buttner et al. (1999) used *P. chrysogenum* in their studies on duct and floor materials, while Kildeso et al. (2003) used *P. chrysogenum* in an experiment to determine the air velocities needed to release spores from wet building materials. Spores from the plates were harvested by flooding dishes with 1 g of 0.5-mm-diameter glass beads (BioSpec Products, Bartlesville, OK) and distilled water, shaking them for 10 s and pouring the suspension into a

sterile vessel containing distilled water. After the beads settled out, the spore suspension was poured through a funnel lined with a polypropylene filter (pore size: 27.0 μm ; US Filter, Siemens, New York, NY) and into a final vessel. A similar harvesting technique was utilized by Schmechel et al. (2005) and Mainelis et al. (2005). During the trials, a total of approximately 1000 ml of spores in suspension were collected from the dishes, and the suspension was stored at 1.7°C until needed.

2.2.1 Nebulizers

During the experiment, spores in distilled water were aerosolized into the chamber using two Allied Health Care B&F disposable respiratory nebulizers attached to Thompson air pumps (Port Orange, FL) running at a combined flow rate of 10.0 LPM. The nebulizers are jet collision atomizers that produce water vapor droplets with mean diameters of less than 5.0 μm . This method of aerosolization has been used in several studies, including those testing the performance of mold sampling equipment (Aizenberg et al. 2000; Chen and Li 2005) and the ability of HVAC filters to capture spores (Pérez 2004).

2.2.2 Sampling instruments

AGI-30® glass impingers (Ace Glass, Vineland, NJ) connected to Gast vacuum pumps (Benton Harbor,

MI, USA) captured air and spores from the bays at a flow rate of 12.5 l min⁻¹. The air was directed into the impingers' reservoirs, which contained 20.0 ml of distilled water. Once sampling was completed, drops of suspension were removed from the impingers, and spores were enumerated using an AO Spencer Bright-Line hemacytometer (Southbridge, MA, USA) for spore enumeration.

2.3 Experimental procedures

Two experiments were conducted inside the wall as part of this study. Experiment one examined spore migration from the distant bay, with wiring installations in the studs between it and the sampling bay three bays away. Experiment two examined spore migration from the adjacent bay, with no wiring installations, to the sampling bay. The wiring hole in the sampling bay was sealed with expansion foam in the fill hole (shown in Fig. 1) to isolate the bays during Experiment two. A single trial for both experiments consisted of aerosolization, pressurization, sampling, decontamination, and enumeration. Aerosolization, pressurization, and sampling were conducted simultaneously to simulate an airborne spore condition. Each trial lasted for 15 min. Eighteen trials were conducted for each experiment, and a total of 36 trials were conducted ($n = 36$) during the study. The positioning of impingers, nebulizers, and supply lines for both experiments are depicted in Figs. 3 and 4.

Fig. 3 Wall Experiment one

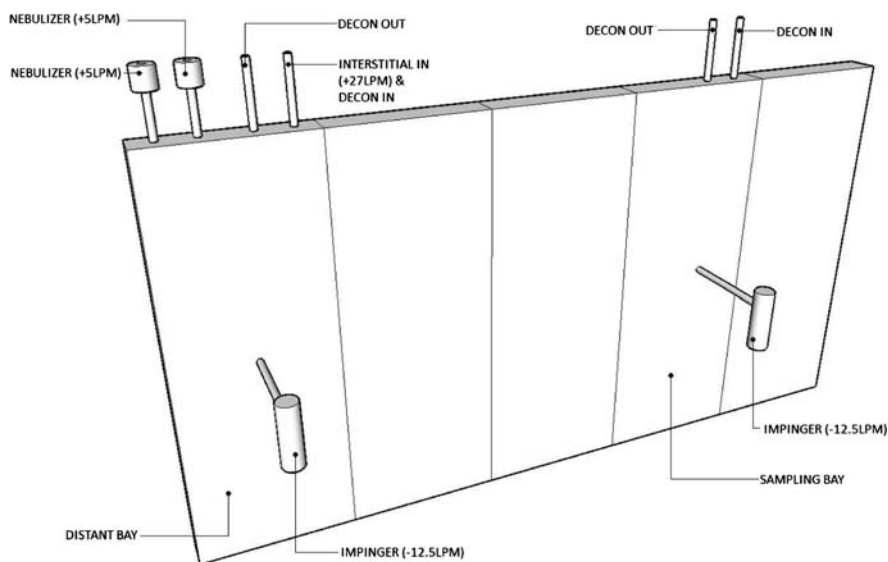
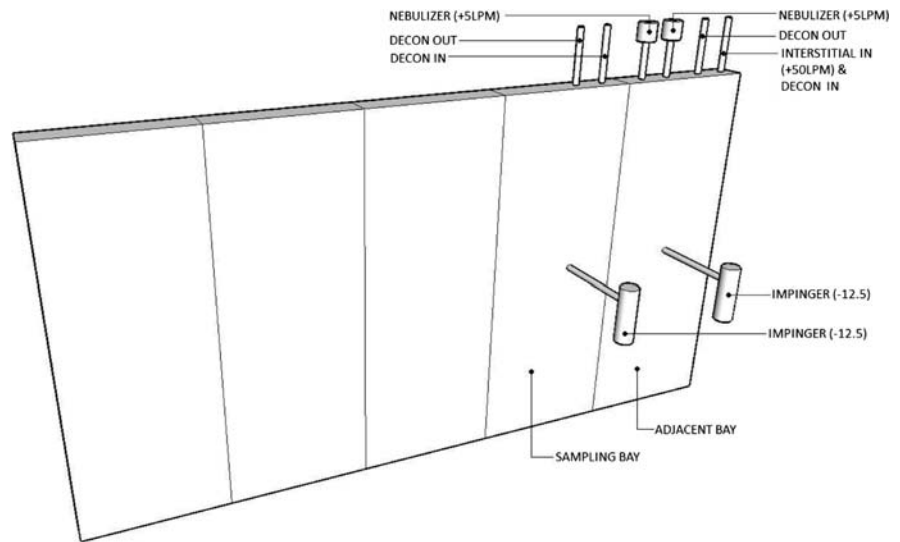


Fig. 4 Wall Experiment two



2.3.1 Aerosolization

Two nebulizers were filled with 15 ml of spore suspension, attached to two elbow conduits leading into the distant bay in Experiment one and into the adjacent bay in Experiment two. The airborne spores were mixed with the air flow paths entering the cavity. A constant supply was made available by running the nebulizers and pressurizing the cavity simultaneously.

2.3.2 Pressurization

During the experiments, a Cable Porter 150PSI air compressor (Delta/Porter Cable, Jackson, TN) subjected only the distant bay and adjacent bay to 20 Pa of pressure; this was called interstitial air. Flow requirements for the impingers and nebulizers had no influence on interstitial air delivered to the distant bay in Experiment one. However, the impinger at the sampling bay in Experiment two did generate a slight vacuum of -5.0 Pa. on the adjacent bay. Subsequently, 25% of the air leakage through to the sampling bay was driven by the sampling pump, and interstitial air simulations in the wall were considered to be mechanically induced pressure changes for this study. Pressure in the bays was verified during trials with a Dwyer magnehellic gauge (Chicago, IL) and flow rates.

2.3.3 Sampling

During Experiment one, impingers were positioned outside the center of the distant bay and the sampling bay. During Experiment two, the impingers were positioned outside the center of the adjacent bay and the sampling bay. The impingers were fitted with 1/4-inch plastic tubing and connected to 1/4-inch plastic hose barbs screwed into the wall. Two bays were sampled simultaneously during trials for each experiment at a rate of 12.5 LPM. An impinger was also used to collect 15-min background samples and 4-min decontamination samples in the bays between trials.

2.3.4 Enumeration

Two aliquots of suspension were removed from the impingers and placed into the AO Spencer Bright-Line hemacytometer counting chamber for spore enumeration under a microscope equipped with a $25\times$ objective and $10\times$ eyepieces using the procedure recommended by the AO Spencer Bright-Line user's manual (American Optical 1974). Denser samples from the nebulizer were enumerated from digital hemacytometer images using a high resolution 3.0 megapixel digital scope camera with Microtec (Niton, Japan) software. A minimum of 30 spores were required to appear on the hemacytometer grid in order to be able to reliably differentiate spore concentrations between the bays. Pre-trial tests were conducted

to adjust nebulizer spore concentrations and nebulizer run-times so as to satisfy this requirement. A concentration of at least 350,000,000 spores 30 ml^{-1} in the nebulizers and a run-time of at least 15 min were established as the minimum conditions to allow at least 30 spores to appear on the hemacytometer grid after migrating through the adjacent bay.

2.3.5 Decontamination

An air compressor, operating at 120 psi was used to flush the interior of all wall stud bays for 20 s after each trial. Decontamination exit lines on each stud bay were opened during the flushes, and air was directed to a ventilation duct. The pipette and hemacytometer were also rinsed with sterile water between trials. Decontamination spore levels inside the pair of bays during each experiment were averaged for the analysis.

2.3.6 Statistical analysis

Statistical analyses for these experiments were performed using XLStat (New York, NY). Differences in spore counts between the bays were evaluated using the Kruskal–Wallis and Dunn's post hoc tests.

3 Results

Kruskal–Wallis tests were conducted to evaluate spore level differences between bays during both experiments. Background levels and averaged decontamination levels were also included in these analyses. Post hoc comparisons were conducted using Dunn's test. The test for Experiment one was significant [χ^2 (df = 3, n = 18) = 54.044; P < 0.05]. Post hoc testing revealed that background and decontamination spore levels were significantly lower than spore levels in the distant bay and sampling bay; however, they were not significantly different from each other. Spore levels inside the distant bay were not significantly different than those in the sampling bay. The test for Experiment two was also significant [χ^2 (df = 3, n = 18) = 57.422; P < 0.05]. Post hoc testing revealed that spore levels inside the sampling bay were significantly lower than those in the adjacent bay. Decontamination and background spore levels were once again significantly lower than the

those in the sampling bay and adjacent bay but not significantly different from each other. Box plots of spore concentrations in both experiments are shown in Figs. 5 and 6. Table 1 compares the average spore

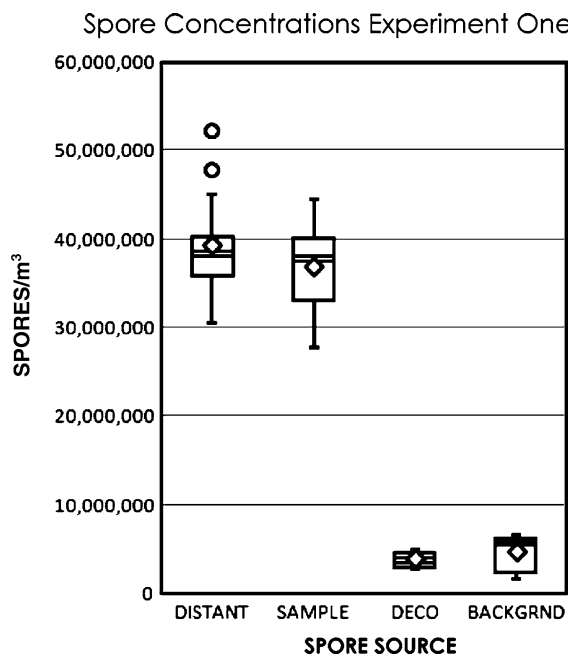


Fig. 5 Spore concentrations: Experiment one. *DECO* decontamination, *BACKGRND* background

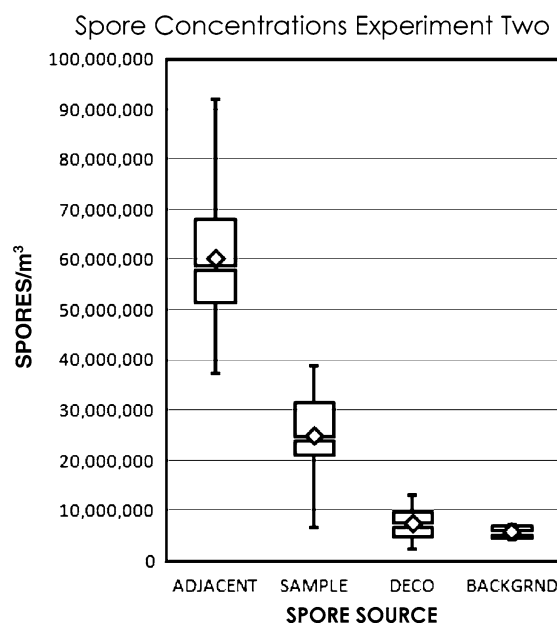


Fig. 6 Spore concentrations: Experiment two

Table 1 Cavity pressures and average spore levels

Experiment	Pressure/spores	Distant bay	Sampling bay	Adjacent bay
One	Pressure (Pa)	20	20	–
	Spores (millions m ⁻³)	37 ± 5	39 ± 4	–
Two	Pressure (Pa)	–	10	20
	Spores (millions m ⁻³)	–	25 ± 9	60 ± 14

Spore concentration is given as the mean ± standard deviation

–, Not measured during these trials

levels and pressures in the bays during the experiments. In Experiment one, the average pressure inside the sampling bay and distant bay was equal, and mean spore levels were similar. In Experiment two, the average pressure in the adjacent bay was twice as high as that in the sampling bay, and average spore levels in the adjacent bay were roughly twice as high as those in the sampling bay.

4 Discussion

The results of Experiment one revealed that airborne *P. chrysogenum* spores were able to move through three simulated wall stud bays with wiring installations when driven by mechanically induced interstitial pressure. Spore levels in the sampling bay were not significantly different from levels in the distant bay after 15 min, suggesting that spores migrated through the wiring orifices in the studs and reached a steady-state across all bays. This state may have been attributed to a pressure equalization that eventually occurred in the bays. With initial changes in the air pressure, air velocities may have carried spores from the distant bay through the wiring orifices until they reached the sampling bay. Once all the bays were equalized, the spores mixed evenly across the bays. The results of Experiment two suggest that while pressure equalization and spore steady-state were not established between the adjacent bay and the sampling bay, airborne mold spores did penetrate through a single stud bay without a wiring orifice under a mechanically induced interstitial pressure. The spore movement and the slight pressurization of the sampling bay may have occurred due to small crevices between the wood stud and gypsum wall board. These crevices exist because commercially available wall studs are slightly warped

or twisted and do not allow a perfect seal when the wall board is attached. Overall, spore migration may have been a function of air pressures inside the stud bays because pressure levels were similarly proportional to spore levels in both experiments. This finding is in agreement with Mosley et al. (2001) and Airaksinen et al. (2003) who determined that penetration factors for particles with diameters similar to those in our experiments were dependent upon pressure differences. Their analysis also showed that the bays were properly decontaminated between trials and that background spore levels did not influence spore levels inside the bays.

Our experiment is not without limitations. The study only examined *P. chrysogenum* spore penetration and, therefore, the results cannot be generalized for all molds. Spore deposition velocities, multiple spore sizes, and spore loss to wall components were not examined as part of this study. The aerosol generated by the nebulizer did not undergo diffusion drying or electrical neutralization before entering the cavity, and this may have contributed to additional spore losses. The experiment also assumed complete spore mixing had taken place inside stud bays. Interstitial air pressures do not always impact a single bay as simulated in this experiment. Other walls can be subjected to lower pressures from mechanical ventilation or stack effects, and results using these pressures may have been different. Wiring installations in wall studs are not consistent, and such variability may also yield different results.

5 Conclusion

The purpose of this study was to determine if airborne mold spores have the potential to move laterally across interior wall stud bays under

pressures typical of those found indoors. The results from the experiment provide evidence suggesting that certain airborne spores can move laterally through wall stud bays with wiring installations and may move through stud bays without wiring installations. Spore movement was driven by velocities generated from mechanically induced pressures that carried spores through the bays. Findings from the experiment may help to explain how mold contamination in a wall can spread from one wall to other more vulnerable areas of the building envelope. Additional research is needed to determine if interstitial pressure changes can liberate enough spores to allow lateral airborne spore movement and to determine the maximum number of bays that could be impacted from such movement.

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