FACTORS RELATING TO THE RELEASE OF STACHYBOTRYS CHARTARUM SPORES FROM CONTAMINATED SOURCES

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ABSTRACT
Although traditionally, fungi as building contaminants have primarily been viewed as allergens, adverse health effects resulting from inhalation of fungal spores are likely due to multiple factors. One factor is the mycotoxins produced by some fungi. This paper describes the preliminary results of a research project to determine the factors that control the release of *S. chartarum* spores from a contaminated source and test ways to reduce spore release and thus exposure. As anticipated, *S. chartarum* spore emissions from gypsum board at low flow are directly proportional to airflow and indirectly proportional to relative humidity and support our previous observations with *Penicillium* and *Aspergillus*. The relationship between the culturable colony-forming units (CFUs) and total spores varied over time and needs further investigation, but suggests one reason that correlation between airborne field measurements (usually only of culturable organisms) and possible exposure is so difficult.

KEY WORDS
Bioaerosol, chamber study, duct, emissions, microbial contamination

INTRODUCTION
Although traditionally, fungi as building contaminants have primarily been viewed as allergens (and occasionally, pathogens), adverse health effects resulting from inhalation of fungal spores are likely due to multiple factors (Miller 1992). One factor associated with certain fungi is low molecular weight toxins (mycotoxins) produced by these fungi. Traditionally, mycotoxins are important to human and animal health because of their production by toxigenic fungi associated with food and feed. However, mycotoxins tend to concentrate in fungal spores (Sorenson et al. 1987), and thus present a potential hazard to those inhaling airborne spores. Toxigenic spores strongly affect alveolar macrophage function and pose a threat to individuals inhaling mycotoxin-contaminated material. A number of reports have indicated that *Stachybotrys chartarum* and several toxigenic species of *Penicillium* and *Aspergillus* are potentially hazardous. The mycotoxins found in indoor air are most likely contained in the aerosolized spores or spore fragments of the toxigenic fungi (Miller 1992).

This paper describes the preliminary results of a research project to determine the factors that control the release of *S. chartarum* spores from a contaminated source and test ways to reduce spore release and thus exposure. Although there have been numerous reports of *S.*

chartarum growing on a variety of building and structural materials that resulted in contamination of buildings and sick individuals, information on what environmental conditions permitted their growth has been limited. Furthermore, *S. chartarum* can be difficult to isolate in air samples. A key issue is understanding how exposure occurs.

MATERIALS AND METHODS

The experiments were conducted in the Dynamic Microbial Test Chamber (DMTC) (VanOsdell et al. 1996). The DMTC is a room-sized test facility designed and constructed to conduct studies on the conditions and factors that influence biocontaminant emissions and dissemination. The chamber, a cube with inside dimensions of 2.44 m, was constructed with stainless steel walls and floor, and an acrylic drop-in ceiling. Temperature (18 - 32 °C) and relative humidity (RH) (55 to 95%) control is provided through an air handler unit (AHU) with an air circulation rate of 1.4 to 4.8 m^3/min. All air in the DMTC is filtered in a high-efficiency particulate air (HEPA) filter downstream of the cooling coil and contains essentially no particles larger than 1 μm in aerodynamic diameter.

The DMTC was adapted to contain eight room-wall simulators (RWS) in which the *S. chartarum* was allowed to grow and then, with minimal physical disturbance, release spores. Figure 1 is an artist's rendition of the DMTC containing the RWS and the sampling apparatus used to measure spore emissions. For clarity, only three of the eight RWS are shown. The DMTC is used for containment, and as a source of conditioned air for the eight RWS.

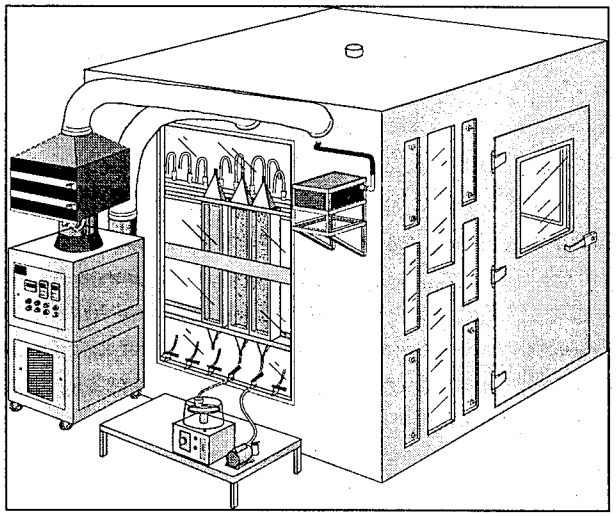

Figure 1. Artist's rendition of DMTC with room-wall simulators (RWS).

The RWS (17.1 cm^2 cross section by 161.7 cm long duct sections) were constructed of 16 gauge stainless steel. The front cover was glass to permit visual monitoring of growth on the test material. The inlet and discharge ends of the RWS transition to 2.5 cm tubes. Each RWS was connected to a pressurized plenum fed by a blower drawing clean conditioned air from the DMTC. A single piece of gypsum board 107 x 42 cm (4494 cm^2) was scored length-wise to permit it to be folded into a three-sided trough that fit into and formed the interior walls of the RWS. Air from the plenum entered each RWS through the inlet transition, flowed down along the gypsum board walls, and discharged through the outlet transition into the DMTC. As shown in Figure 1, bioaerosol samplers periodically drew samples from the air leaving the RWS.

The gypsum board pieces were loaded into the RWS and the entire assembly autoclaved. Each 4494 cm^2 gypsum board piece was wetted with 200 mL of sterile water three times for a total of 600 mL. After each wetting, the water was allowed to soak into the gypsum board. Preliminary experiments showed that the water wicked throughout the entire piece and that a uniform distribution of water over all surfaces was easily achieved. The goal was to simulate a catastrophic wetting of the material. The gypsum board was then inocu-

lated with 12.9 mL of 10^6 CFU/mL *S. chartarum* manually pipetted in 15 µL spots uniformly distributed across the surface of the gypsum board.

The RWS were sealed, and growth was allowed to proceed under static conditions for at least 1 month until relatively heavy growth was visible. The RWS were then mounted on a rack within the DMTC. The air velocity was set to either < 10 or 35 cm/s, on the order of velocities encountered in the conditioned indoor space (ASHRAE 55-1992). The RWS were designed so that the air flowed down to more closely mimic wall contamination.

The temperature in the RWS was maintained at 23.5°C. At the start of an emission rate determination, the DMTC RH was lowered to the test RH (i.e., 64%) by lowering the RH setting on the AHU. The RH change was effective within minutes. Isokinetic air samples were collected using the Mattson-Garvin slit to the agar sampler for culturable fungi and Air-O-Cells (Zefon Analytical Accessories, Saint Petersburg, FL) for total spore counts. Surface samples, collected only at the conclusion of all the air sampling, were obtained by vacuuming 10 cm^2 sections of the gypsum board.

The Mattson-Garvin draws air at 28.3 L/min through a metal inlet with a 0.015-cm slit, allowing the impaction of airborne organisms on the surface of a rotating 150-mm agar plate. The sampler plates were incubated at room temperature. CFUs were counted shortly after visible growth was first noted and again as moderate growth became apparent.

Air-O-Cells are preloaded cassettes containing a glass slide coated with a sticky impaction medium. The base of the cassette was connected to a pump using flexible tubing, and air was drawn onto the impaction surface at 28.3 L/min through a slit in the top of the cassette. Total airborne spores were quantified by opening the Air-O-Cell and removing the internal glass slide containing the impaction medium. The slide was placed onto a microscope slide and stained with lacto-glycerol. Total airborne spores were counted microscopically.

Calculation of Emission Rates

To calculate the emission rates for the culturable organisms, the CFUs on the sampler plates were enumerated, and the CFUs/min were determined. For total spores, the spores/min were calculated. Both values were adjusted for the total flow rate, and divided by the area of the emitting surface.

RESULTS AND DISCUSSION

Previous experiments showed that the spore emission rate for *Penicillium chrysogenum* and *Aspergillus versicolor* were directly related to airflow and indirectly related to relative humidity (Foarde et al. 1999). As the air velocity increased across the surface of the duct, emissions increased. As the RH was lowered, the emission rate increased for both organisms. The first step was to determine whether the spore emission rate for *S. chartarum* would follow a similar pattern.

The earlier work with *P. chrysogenum* and *A. versicolor* was performed under duct flow conditions because these organisms frequently contaminate fiberglass duct liner. Although there are reports on *S. chartarum* growing on fiberglass duct liner, for this series of experiments we selected gypsum wallboard as the test material. The duct airflow levels that

we used previously were not appropriate for wall air flow. For this preliminary work, we selected two air velocities: 10 cm/s, which we consider very low velocity, and 35 cm/s or low velocity.

Table 1 shows the results of a series of experiments at very low and low air velocity in the RWS. The emission rates are expressed as CFU/m^2/min. The first-hour emission rates at each RH are shown in the table and confirm that *S. chartarum* spore emissions increased with increased airflow and as the humidity was lowered.

Table 1. Culturable CFUs in CFU/m²/min emitted at two airflows at four relative humidities.

Airflow, cm/s	Relative Humidity, %			
	95	85	75	65
< 10	0	0.4	25	50
35	4	29	65	200

The second set of experiments were designed to quantify emissions at 65% RH over an extended period. The previous study, with *P. chrysogenum* and *A. versicolor*, showed that emissions continued for at least 17 hours. For *S. chartarum*, emissions were measured until they were below the detection limit (< 5 CFU/m^2/hr).

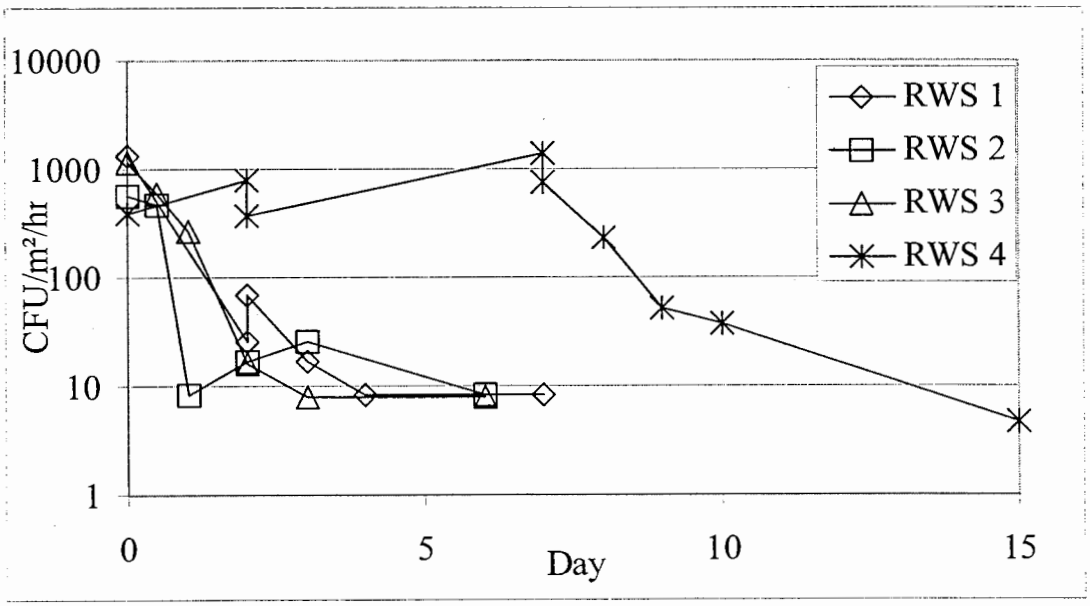

Figure 2. *S. chartarum* CFU emissions from four RWS at 65% RH and 10 cm/s airflow.

Figure 2 shows the results from four RWS. Note that the surface concentrations of the wallboard in the four RWS were essentially the same, ranging from 5 x 10^4 to 5 x 10^5 CFU/10^2 cm^2. What may be more important is the age of the growth on the RWS. RWS 1 through 3 were 2 months old when the emission experiments were initiated, whereas RWS

4 was 3 months old. We are continuing to investigate the surface-to-airborne concentration relationship.

As can be seen in Figure 2 spore emissions from RWS 1 through 3 started at levels between 500 and 1,000 CFU/m^2/hr on day 0 and tapered off by day 2 or 3. RWS 4 started at the same initial level as RWS 1, 2, and 3 but continued to emit that same level of spores for the entire first week before the level started to decrease.

The next set of experiments was designed to investigate the emission of spores at RH below 65%. The low air velocity of 35 cm/s was used for this experiment. The RH was allowed to range from 25 to 64%, the normal range of room humidities in controlled environments.

Figure 3 shows the hourly emission rates of both culturable CFUs and total spores/m^2 of gypsum board from one RWS (3-month age of growth). Culturable CFUs, designated by the diamonds, are plotted on the left y-axis. Total spores are plotted on the right y-axis with squares. Figure 3 shows fairly high levels of emissions, continuing for at least 80 days.

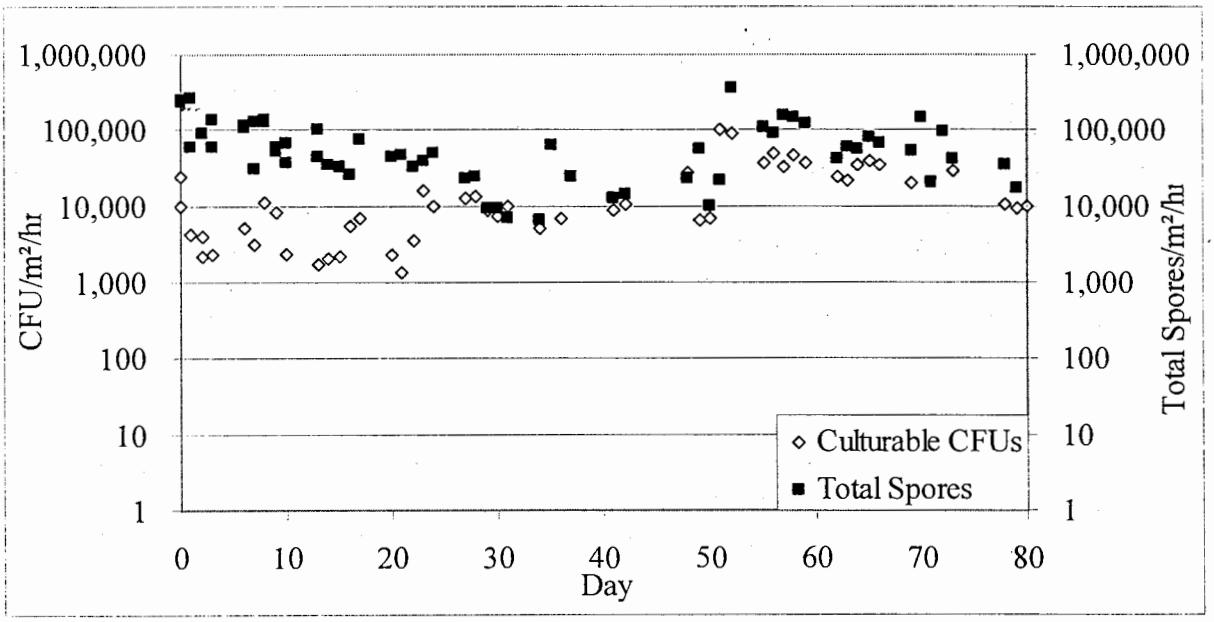

Figure 3. *S. chartarum* spore emissions at RHs between 25 and 64% at low air flow (35 cm/s).

The data show an interesting relationship emerging between the numbers of culturable CFUs and the numbers of total spores enumerated. For the first 30 days, the CFUs appeared to be about 10% of the number of total spores. Then the levels of culturable CFUs and total spores seem to converge. Figure 4 shows the percentage of culturable CFUs to total spores over the nearly 3-month experiment.

Figure 4 shows that, for the first 30 days, the culturable CFUs are approximately 10% of the total spores. For the next 30 days (days 30-60), the percentage of culturable spores in-

creased notably compared to the first 30 days. For the final 25 days, the percentage of culturable CFUs was lower than the second 30 days, but higher than the first 30 days. We are continuing to investigate.

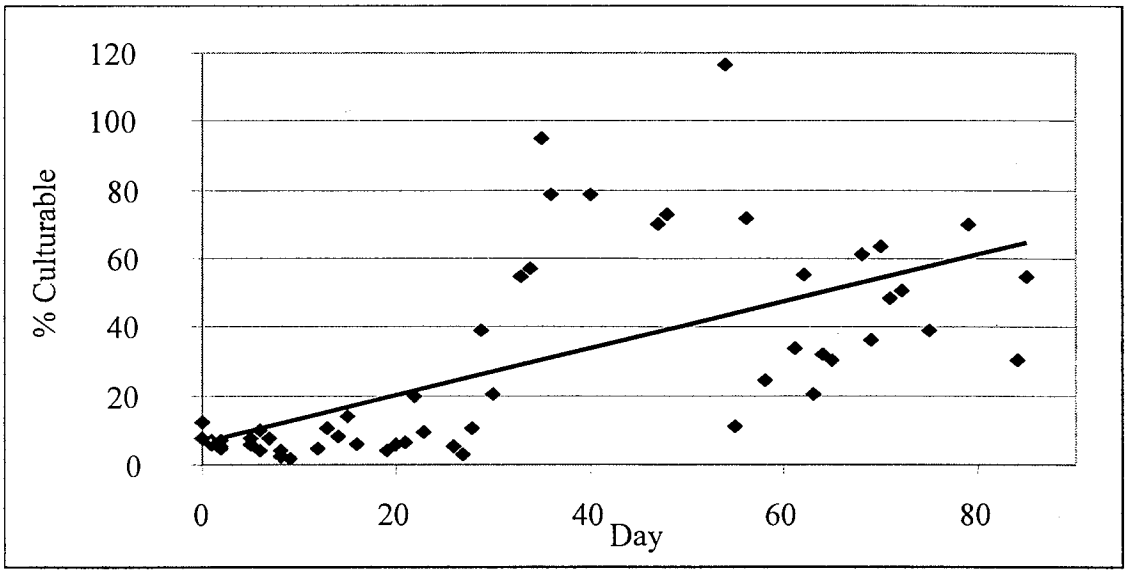

Figure 4. Percent of the total spores that are culturable CFUs.

CONCLUSIONS AND IMPLICATIONS

Significant progress has been made on elucidating release factors and the potential implication for exposure. As anticipated, *S. chartarum* spore emissions from gypsum board at low flow are directly proportional to airflow and indirectly proportional to RH and support our previous observations with *Penicillium* and *Aspergillus*. The relationship between the culturable CFUs and total spores needs further investigation, but suggests one reason that correlation between airborne field measurements and possible exposure is so difficult. Generally, field measurements consist of collecting culturable CFUs. Additional measurements, such as quantifying total spores, are needed.

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17. KEY WORDS AND DOCUMENT ANALYSIS

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Pollution	Pollution Control	13B
Aerosols	Stationary Sources	07D
Spores	Microbial Contamination	06C
Microorganisms	Bioaerosols	06M
Chambers		14G
Ducts		13K
Emission		

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