

Growth Response of *Stachybotrys chartarum* to Moisture Variation on Common Building Materials

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Key Words

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Abstract

The mould *Stachybotrys chartarum* has been found to be associated with idiopathic pulmonary haemorrhage in infants and indoor exposure has also been linked to other pulmonary diseases, including allergies and asthma. *S. chartarum* has been studied both for toxin production and its occurrence in water-damaged buildings. Growth of *S. chartarum* on building materials such as gypsum wallboard has been frequently documented. Given that there may be a high frequency of occurrence and so the risk of exposure, environmental factors leading to the growth of *S. chartarum* have been studied. Samples of commonly used building materials were sterilised, inoculated with *S. chartarum* and exposed to controlled levels of relative humidity and wetting. A quantitative analysis of viable *S. chartarum* was performed on the building materials during a 7-month period. The results indicate that for environments with a relative humidity below total saturation, wetting was necessary for visible growth to occur.

Conversely, high levels of relative humidity without wetting did not initiate growth. Porous materials, after becoming sufficiently wet and measuring saturation on a moisture meter, exhibited mould growth in every experiment conducted.

Introduction

The past 20 years have brought the recognition that an important factor in the health of people in indoor environments is the dampness of the buildings in which they live and work [1-3]. Furthermore, it is now appreciated that the principal biological organisms responsible for the health problems in such buildings are fungi rather than bacteria or viruses [1-4]. Although traditionally fungi in this context have been viewed as a source of allergens (and, in unusual circumstances, pathogens), data have accumulated to show that the adverse health effects resulting from inhalation of fungal spores are due to multiple factors [5]. One such factor, associated with certain fungi, is the low molecular weight toxins (mycotoxins) they produce. Mycotoxins are held to be important in human and animal health because of their production

by toxigenic fungi associated with food and animal feed. The problem is compounded since mycotoxins tend to concentrate in fungal spores and thus present a potential hazard to those exposed who inhale them. Toxigenic spores strongly affect alveolar macrophage function. Reports have indicated that *Stachybotrys chartarum*, *Aspergillus versicolor* and several toxigenic species of *Penicillium* are potentially hazardous, and exposure may occur when the air-handling systems in a building have become heavily contaminated [3–6].

One of these toxigenic fungi, *S. chartarum*, found in wet buildings, is known to produce the very potent cytotoxic macrocyclic trichothecenes along with a variety of immunosuppressants and endothelin receptor antagonists mycotoxins [1–3]. Infants have been admitted to Case Western University Hospital in Cleveland in a very grave condition expelling blood from their noses and mouths due to Pulmonary Haemorrhage (PH) [1–3]. There have been 45 cases of PH in young infants, of whom 16 have died. Most of these cases have occurred within an area of 10 contiguous zip codes in the eastern portion of the metropolitan area. In November/December 1994, the Centers for Disease Control and Prevention (CDC) led a case-control investigation on the first 10 cases. This study found an epidemiological association of PH in these infants with water-damaged homes containing toxic fungi, predominantly *Stachybotrys* [1–3]. The importance of environmental tobacco smoke exposure in conjunction with *Stachybotrys* to produce PH has been discussed by Sudakin (2000) [5]. However, its importance as a multiplicative risk factor for toxic mould exposure and PH is unknown.

Stachybotrys requires water-soaked cellulose to grow and has been found in homes where there has been water damage from flooding, plumbing leaks, or roof leaks involving wood or paper products (e.g. insulation, gypsum board, ceiling tiles). The spores of this fungus contain mycotoxins which appear to be particularly toxic to the rapidly growing lungs of infants [1–6]. Although not widely found, *Stachybotrys* has been studied for the last 20 years. The following is documented: (1) *S. chartarum* produces toxigenic spores that are potentially hazardous, (2) the prevalence of *S. chartarum* contamination in indoor environments is unknown, (3) currently there are no Environmental Protection Agency (EPA) regulations or guidelines for evaluating potential health risks of *S. chartarum* contamination and remediation, and (4) the exact environmental conditions necessary for the growth of *S. chartarum* have not been documented [1–6]. Beyond the recorded instances of *S. chartarum* growing

in moisture-laden buildings, the exact required levels of Relative Humidity (RH) are not known. This paper presents the findings of a series of experiments in which *S. chartarum* was grown at different RH levels, wetting regimes, and on several building materials.

Materials and Methods

Gypsum Board

Experiments were conducted at room temperature (21.1°C (70°F)) using regular gypsum wallboard composed of a gypsum (calcium II sulfate, dihydrate) core wrapped with paper. The four main material types used were: (1) new gypsum board, (2) old gypsum board, (3) new gypsum board with vinyl-coated wallpaper applied to the top surface, and (4) new gypsum board with 100% vinyl wallpaper applied to the top surface.

Regular gypsum wallboard, vinyl-coated wallpaper, and 100% vinyl wallpaper were purchased from local vendors, and old gypsum board was donated for the experiment. For both the new and the old gypsum board experiments, the materials were cut into 3.8 × 3.8 cm (1.5 × 1.5 in) squares, placed in self-sealing pouches, and steam sterilised by autoclaving. The pieces were then removed from the pouches and inoculated with 50 µL of *S. chartarum* spores, resulting in approximately 10⁵ colony forming units (CFUs) per test piece. When the spore inoculum was dry, the pieces were placed into the appropriate static chamber, depending on the relative humidity at which the experiment was being conducted [7]. The static chamber testing was based on ASTM Standard D6329-98. A static chamber is an acrylic-walled desiccator with a tray containing a saturated salt solution on the bottom. The salt solution maintains the desired RH [7].

The gypsum board was cut in large sections, and the sections were covered with vinyl-coated wallpaper and 100% vinyl wallpaper. The sections of gypsum board were autoclaved, allowed to dry, and then inoculated. After the inoculum was dry, the wallpaper was applied over the inoculated gypsum board with sterile water. When the wallpaper was dry, the pieces were cut into 3.8 × 3.8 cm (1.5 × 1.5 in) squares and placed in appropriate static chambers.

The inoculated pieces of gypsum board were tested under many different conditions to assess their ability to support *S. chartarum* growth. New gypsum board pieces were tested at 85, 90, 97 and 100% RH. Further experiments were conducted at 62 and 75% RH where 1 mL of sterile water was added to each piece prior to inoculation, as well as experiments at 62, 75, 85, 97 and 100% RH where 4 mL of sterile water was added to each piece.

For the old gypsum board experiments, pieces were incubated at 85, 90, 97 and 100% RH. One experiment was also conducted at 100% RH with 4 mL of sterile water added to each piece prior to inoculation with *S. chartarum*. All experiments with both types of wallpaper were conducted at 85, 90, 97 and 100% RH.

To minimise error and demonstrate reproducibility, 30 pieces of each type of material were treated and inoculated on day 0. Of these, three pieces of each material type were sampled on their respective sampling days. To determine how many organisms

were on the materials, the samples were removed from the chamber, placed into separate sterile containers with 30 mL buffered detergent, and shaken vigorously for 30 min to elute the microorganisms. The sample/buffer suspension was then diluted as necessary and plated on Sabouraud's dextrose agar. The plates were incubated at room temperature, and CFUs were counted when moderate growth became visible [8]. For each experiment, samples were processed and analysed at day 0, and in some cases biweekly or monthly up to 7 months of incubation, depending on the length of time needed for the initiation of growth.

The experiments with new gypsum board were further subdivided to determine if the Research Triangle Institute (RTI) field isolate of *S. chartarum* and two other strains of *S. chartarum* behaved similarly under varying conditions. Of the two additional strains, one was labeled as being high in toxicity, and one was labeled as being low in toxicity. Experiments were conducted with the RTI isolate at 85, 90, 97 and 100% RH. Four millilitres of sterile water was added to some pieces before they were incubated at 62, 75, 85, 97 and 100% RH. The pieces inoculated with the high and low toxicity *S. chartarum* strains were incubated at 97 and 100% RH, as well as at 62 and 75% RH after 1 and 4 mL of sterile water were added. All pieces were processed and analysed in the same manner as described above.

Gypsum Board Components

Additional experiments were performed on the individual components of the gypsum board. Square 3.8×3.8 cm (1.5×1.5 in) pieces were divided into the top and bottom papers and the core. The core was wetted with 1 mL of sterile water, and the papers were individually wetted with 0.5 mL sterile water prior to inoculation with *S. chartarum*. They were incubated at 100% RH in a static chamber and processed in the same way as the other gypsum board samples.

Other Materials

Wood, masonite and cellulose insulation were also inoculated with *S. chartarum* and placed in static chambers. The wood materials and masonite were subjected to three different wetting scenarios. They were all cut into smaller pieces and, after being autoclaved, soaked in sterile water for 6 days and then inoculated. They were then all placed into 100% RH static chambers. Pieces were also placed into 100% RH static chambers without wetting, and some types of materials were wetted with 1 mL of sterile water before inoculation and incubation in 100% RH chambers.

The cellulose insulation used is made with 100% recycled paper and is specified as non-flammable. It is classified following ASTM-C739-97 as having an acceptable fungal resistance, a density of 1.6 pound per cubic foot (PCF), and a thermal resistance of 1.5 R/cm (3.7 R/in). The insulation was autoclaved, then divided into equal pieces of 1.5 g (dry weight) and wetted with 15 mL of sterile water. The pieces were then inoculated as described for all of the other materials and placed into a 100% RH static chamber.

All materials were processed and analysed as described for the gypsum board, with varying amounts of sterile buffer depending on the absorption of the buffer by the material. A square

3.8×3.8 cm (1.5×1.5 in) piece of gypsum board would absorb 4 mL of water within a few minutes and would measure as 100% saturated with a moisture meter.

Results and Discussion

The analytical results listed in Tables 1–3 were derived by a quantitative evaluation of the numbers of viable spores found. The growth response is represented by the difference in spore concentration between the start and end of the experiment. The log mean of spore concentration at the time of material inoculation was subtracted from the final log mean concentration. An increase in concentration is signified as a positive number to indicate growth. A decrease in spore concentration is signified as a negative number to indicate die off. A one log increase or decrease was viewed as significant. If the experiment was not conducted, ND is listed in the table.

Three isolates of *S. chartarum* were used in this series of experiments, and were identified as RTI isolate, low toxicity and high toxicity. In the work addressed in Table 1, the RTI isolate was used; Table 2 used all three isolates; and Table 3 used the low toxicity isolate.

The comparison of *S. chartarum* growth results for gypsum board is listed in Table 1. All four configurations of gypsum board tested at 100% RH indicated positive for growth. The gypsum board which had 4 mL of water added tested positive for growth at all RH levels, which varied as low as 62% for this experiment. The one variety of gypsum board which was tested at 62 and 75% RH with 1 mL of water indicated a negative change in concentration, or die off, of spores. One variety of vinyl coated wallpaper showed significant growth at 97% RH without any additional water and was the sole exception. Of the remaining 11 of the total 24 separate experiments showed no change in spore concentration.

The results indicate that at 100% RH, saturated air will promote the growth of *S. chartarum* whether or not there is direct contact with water. However, below 100% and greater than 62% RH, an additional 4 mL of water was necessary for growth to occur. Less additional water at 75% RH caused no change in spore concentration. At 62% RH, 1 mL of water was not effective in promoting growth, and the results indicated a die off in spore concentration instead.

Results listed in Table 2 use three separate isolates to compare 21 sets of growth experiments of different RH and wetting amounts. The results in Table 2 for all the three isolates of *S. chartarum* agree with the results of

Table 1. Gypsum board *Stachybotrys chartarum* growth (see text for explanation)

Material	Static chamber RH									
	62%+ 1 mL water	75%+ 1 mL water	62% + 4 mL water	75% + 4 mL water	85% + 4 mL water	85% 90%	97% 97%	97% + 4 mL water	100% 100%	100% + 4 mL water
New gypsum board	-2.01	-1.83	+3.52	+3.28	-0.06	+2.26	-0.23	-0.07	+2.42	+1.84
Old gypsum board	ND	ND	ND	ND	-0.37	ND	-0.32	+0.58	ND	+1.88
Vinyl-coated wallpaper	ND	ND	ND	ND	-0.13	ND	-0.13	+1.87	ND	+4.55
100% Vinyl wallpaper	ND	ND	ND	ND	-0.80	ND	-1.85	+0.70	ND	+1.49

- = decrease in CFUs (die off); + = increase in CFUs (growth); ND = Not Conducted.

Table 2. *Stachybotrys chartarum* growth isolate comparison

	Static chamber RH											
	62% + 4 mL water	62% + 1 mL water	62% 62%	75% + 4 mL water	75% + 1 mL water	75% 75%	85% 85%	85% + 4 mL water	90% 90%	97% 97%	97% + 4 mL water	100% 100%
RTI isolate	+3.52	ND	ND	+3.28	ND	ND	-0.06	+2.26	-0.23	-0.07	+3.32	+1.84
Low tox	+2.30	-2.01	ND	+2.16	-1.83	ND	ND	ND	ND	-2.40	ND	+1.33
High tox	ND	-2.71	ND	+2.26	-1.10	ND	ND	ND	ND	-2.09	ND	+2.91

- = decrease in CFUs (die off); + = increase in CFUs (growth); ND = Not Conducted.

Table 1. Note that the results of Table 2 indicate that, at 62% RH and 4 mL of water, growth is elicited; but at 62% RH and 1 mL, die off occurred. The difference of 3 mL of additional water absorbed by the gypsum board and incubated at 62% RH was the difference between growth and die off. For all samples receiving 4 mL of water, a positive growth response was recorded, in agreement in Table 1.

Results listed in Table 3 compare 13 sets of growth experiments. A marginally positive growth response was recorded for the 2 × 4 and 1 × 4 wooden boards and a significantly positive growth response for plywood boards that were all soaked in sterile water for 6 days and incubated at 100% RH. The wood board and masonite samples which were wetted and incubated at 100% RH did not grow *S. chartarum*, indicating that significant moisture exposure for a prolonged time period was required.

A positive growth response is listed in Table 3 for cellulose insulation that was wetted and incubated in 100% RH. The material used had been treated with a flame retarding borate solution that was (reported by the manufacturer) to be anti-fungal. However, although it was classified, following ASTM-C739-97, as having an acceptable fungal resistance, a density of 1.6 PCF, and a thermal resistance of 3.7 R/in, *S. chartarum* was able to grow. The

Table 3. *Stachybotrys chartarum* growth on building materials

Material	Test condition		
	100% RH	wetted + 100%RH	soaked + 100%RH
2 × 4 stud	-0.88	-2.16	+0.86
1 × 4	-2.16	ND	+0.96
Masonite	ND	-1.01	-0.87
Plywood	-2.37	ND	+2.75
Cellulose insulation	ND	+5.01	ND
Gypsum board front paper	ND	+2.32	ND
Gypsum board back paper	ND	+2.52	ND
Gypsum board core	ND	+2.66	ND

- = decrease in CFUs (die off); + = increase in CFUs (growth); ND = Not Conducted.

widespread use of cellulose insulation in houses makes this result important and troubling.

As listed in Table 3, the gypsum board components (core, front and back paper) had positive growth responses to wetting and incubation at 100% RH, in agreement with results shown in Table 1 for intact gypsum board. This indicates that growth is not localised or dependent on the paper. *S. chartarum* was able to grow when no paper was present.

Conclusion

Stachybotrys chartarum will grow in saturated air conditions (100% RH) without any additional moisture. At an RH of 97% and below, wetting is necessary for the growth of *S. chartarum*. Variations in the type of gypsum board did not effect growth responses at the levels of RH tested.

All gypsum board samples receiving 4 mL of water on a 3.8 cm (1.5 in) square and incubated at 64% RH or greater were positive for mould growth. This indicates that gypsum board which becomes saturated (as specified by a moisture measurement) and allowed to stay in a marginally humid environment will result in mould growth. Although gypsum board measuring 100% moisture content (saturated) is not noticeably different in appearance from gypsum board with no measurable moisture, it is highly susceptible to mould growth.

Growth responses to various levels of RH were consistently similar for the three strains of *S. chartarum* tested. These strains could be expected to behave similarly at varying RH levels.

Wood boards and plywood that have been soaked in water and allowed to be exposed to saturated RH conditions can grow *S. chartarum*.

Cellulose insulation that has become wet and allowed to be exposed to saturated RH conditions can grow *S. chartarum*. The use of cellulose insulation in walls and ceilings can make many homes susceptible to moisture influx and ultimate mould growth.

Manufactured gypsum board which becomes wet and allowed to remain wet for a protracted length of time is susceptible to mould growth regardless of vinyl covering.

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