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Sanitation of Wallboard Colonized with *Stachybotrys chartarum*

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Abstract. Sections (8 cm^2) of unused, nonsterile gypsum wallboard (dry wall) were inoculated with varying densities (10^4 to $\sim 10^8$/ml) of conidia from 14- to 21-day cultures of *Stachybotrys chartarum* grown on cellulose agar. The sections were permitted to air dry and were placed into vessels with 86% or 92% RH and incubated at 22–25°C for up to 12 weeks. The moisture content of the dryboard increased from near 10% to over 35%. Selected sections with confluent surface growth, mainly of *S. chartarum*, were obtained within 3 weeks. Sections were cleaned with a quaternary or quaternary and chlorine dioxide or a concentrated oxygen-saline solution and treated, in some cases, with a preservative system and returned to humidity vessels. Reemergence of *S. chartarum* from inoculated and treated surfaces occurred within 5 weeks only with sections treated with the quaternary alone. Other fungi, mostly species of *Aspergillus*, *Chaetomium* and *Penicillium*, slowly colonized (between 9–12 weeks) at least some areas of most treated surfaces and most uninoculated control surfaces. *Stachybotrys chartarum* was also found on several sections of uninoculated controls. Sections treated with a quaternary/acrylic and placed in a dynamic challenging chamber remained visually free of colonized fungi for over 90 days. These studies indicate that control samples of uninstalled wallboard, available from local distributors, can contain a baseline bioburden, including *S. chartarum*, that will colonize surfaces under high humidity conditions. Sanitation and preservation treatment of the wallboard can markedly delay regrowth of these fungi, particularly of *S. chartarum*.

Stachybotrys chartarum (syn. *S. atra*) is a widespread saprophytic fungus that gained notoriety in eastern Europe in the 1930s as a cause of stachybotryotoxicosis of horses after ingestion of moldy hay. The typical form of fatal stachybotryotoxicosis in animals is characterized by leukopenia, severe thrombocytopenia, hemorrhage, and arrhythmic heartbeat. The species produces a variety of mycotoxins including macrocyclic tricothecenes, and trichoverrols A and B [3, 4]. The toxigenic tricothecenes may be produced (by at least some strains) with growth on cellulosic substrates, including indoor construction and finishing materials such as gypsum wallboard and ceiling tiles [2, 6]. The species has been associated with building-related illness [2, 9] and most recently with fatal hemosiderosis of newborns, but causality of toxic effects on humans via inhalation of toxic conidia is not available [4, 5]. The species is not known to be invasive.

Recommended remediations for ceiling tiles or wallboards colonized extensively with *S. chartarum* usually involve replacement of materials with processing under protective measures [1]. A problem is the definition of ''extensive'' colonization and whether areas greater than 3 m^2 of surface area with scattered growth and no marked structural alteration present a greater or lesser problem than lesser areas with more intense growth. A single microcolony of *S. chartarum* on a wallboard could yield more than one colony-forming unit per gram of bulk sample of wallboards (a guideline limit). Strict adherence to such guidelines therefore, could involve unnecessary containment procedures for remediation. Traditional mold clean-up procedures involve washing the affected area with a household bleach solution (one cup per gallon of water) that remains in contact with the surface for at least 15 minutes. In certain confines even this procedure is objectionable because of the odor.

This research is a laboratory-based study of the efficacy of several sanitization systems for gypsum wallboard colonized with *S. chartarum*.

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Fig. 1. Moisture chambers with wallboard samples.

Materials and Methods

Dry wall samples (gypsum board). Two sheets of dry wall were obtained from separate outlets of a building supply firm. The dry wall consisted of a ⅜ inch layer of gypsum covered with a finish side (smooth) paper and a cavity side (rough, kraft) paper. The dry wall was cut into sections (about 8 × 8 cm).

Stachybotrys chartarum, initially isolated from a ceiling tile, was grown in 100-mm petri dishes on cellulose agar (KCl 0.5 g, $MgS0_4$ 0.5 g, KPO_4 1.0 g, $NaNO_3$ 2.0 g, cellulose powder 20.0 g, H_20 1000 ml) for 14–21 days at 30°C. Plates were flooded with sterile saline with 0.1% Tween 80, and conidia were harvested from the entire surface area with a sterile cotton swab. The conidial suspension was filtered through glass wool, and pooled suspensions were placed in a sterile 50-ml conical tube. This resulted in a very dark melaninated suspension that contained from 10^7 to 10^8 conidia/ml. This stock solution of conidia was diluted to 10^4 and 10^6/ml and used within 48 h of preparation.

A sterile cotton swab was saturated in the conidial suspension (excess fluid expressed) and swabbed over the entire paper facing of both sides of the dry wall samples (two to four replicates for each test preparation). The percentage water content on the swabbed surface immediately after inoculation was from 13% to 15%.

Moisture chambers. Polycarbonate jars (1.0 L, Nalgene, Rochester, N.Y.) with threaded tops were used as moisture chambers (250 ml of deionized water was placed in the bottom of each jar, and a plastic cup with the bottom removed was inverted and used as a pedestal to hold the specimen above the water). Each wallboard sample was placed onto the cup, and the lid of the jar was sealed. The jars were placed initially into a 37°C incubator for 24 h and then removed and placed on a laboratory bench top at room temperature (~24°C). This facilitated the building of moisture in the head space of the jar and condensation on the walls. Each jar was incubated until near confluent growth was established on the paper facing on both sides of the dry wall. The lids of representative jars were equipped with a portal that permitted measurement of the relative humidity with a hygrometer (Rosemount, Analytical Inc., Irvine, CA; Fig. 1). The probe that measured relative humidity and temperature was permitted to equilibrate for 20 min prior to measurements. Moisture content of the wallboards was determined on samples placed in plastic freezer storage bags and read with a non-intrusive moisture meter through the plastic cover (No. M905, Professional Equipment, Hauppauge, NY).

Sanitation procedures. All sanitization procedures were conducted under a biosafety hood with gloved hands. Samples were removed from the moisture chamber jars and cleaned with a round bottle brush with sanitizing chemicals with a 15-min wet contact time. A cleaner (A) consisting of 0.036% didecyl dimethyl ammonium chloride and 0.023% n-alkyl dimethyl benzyl ammonium chloride at use dilution (Allstar Corp., Valley Forge, PA); (B) Cryocide 20 consisting of 0.72% chlorine dioxide and 0.4% didecyl dimethyl ammonium chloride at use dilution (Engelhard Corp., Islen, NJ) and BIO_2 (BIO_2 International Inc. San Luis Obispo, CA); (C) a stabilized high-oxygen-content saline solution, were used as sanitizers. The colonized wallboard sections were placed on a 40° angle in a collection pan. A 5.0-ml volume of sanitizing solution was flooded over the surface of the section. The section was brushed (soft bristle bottle brush for 30 s) and flushed with an additional 5.0 ml of solution. An additional light brushing was followed with a final 5.0-ml rinse. The rinsate was processed for surviving fungi. To determine further the antimicrobial efficacy of these solutions, we exposed conidial suspensions to the working sanitizing solutions for varying time periods followed by dilution of the conidia in enrichment broth with recovery attempts on Letheen agar (BBL, Cockeysville, MD).

Remedial treatments. The wallboard sections, immediately after sanitization, were air dried for 18–20 h under a laminar flow hood. Two types of commercially available antimicrobial coatings were examined. The first coating was a clear acrylic designed to coat aluminum air conditioner heat exchange coils (Airsept Inc., Smyrna, GA). The second coating was a white pigmented, water-based paint designed as a metal priming paint (Porter Paints, Louisville, KY). Both systems contained Intersept (Interface Res., Kennesaw, GA), an amine-neutralized phosphoric acid ester biostat. The thinner clear acrylic-based coating was spray applied to the dry wall surface, while the more viscous metal priming paint was applied by brush.

Sample re-exposure to humidity. After each sample that had been colonized with *S. chartarum* was sanitized (other than controls) and permitted to air dry, the samples were returned to moisture chambers and monitored weekly for at least 5 weeks for the reappearance of

Table 1. Recovery of *Stachybotrys chartarum* and other fungi from wallboard 5 weeks after sanitization and treatment[a]

No. samples	Sanitization treatment	Remedial treatment	Recovery of fungi[b]	
			S. chartarum	Other fungi[c]
8	saline	none	2	8
4	Quaternary (A)	none	4	4
2	BIO_2 (C)	none	2	2
2	BIO_2 (C)	acrylic	(0)[d]	(2)
4	Quaternary (A)	acrylic	0	4
4	Quaternary (A)	primer	0	(4)
4	Quaternary + ClO_2 (B)	acrylic	0	(4)
4	Quaternary + ClO_2 (B)	primer	0	(4)

[a] All wallboard sections were covered with confluent growth of *S. chartarum* prior to sanitization.
[b] No. of samples positive for fungal colonization within 35 days.
[c] Fungal colonization by other than *S. chartarum* observable within 35 days; (0) visually observable only after 35 days.
[d] (0) No recovery of *S. chartarum* at 35 days, but positive recovery on one section after 90 days.

growth. Control sections (8) were swabbed with sterile saline and placed directly into moisture chambers without treatments. Several samples were placed for an additional 60 days into a secondary challenge chamber described earlier [8].

Results

All eight uninoculated and untreated control gypsum wallboard samples incubated at relative humidities from 86% to 96% yielded mainly *Penicillium* spp. and *Chaetomium* spp. on the finished paper side, whereas the kraft paper side more frequently yielded *Aspergillus* spp. and *Cladosporium* spp. Growth was not confluent and often was not observed with the unaided eye until after 4 weeks of incubation. Sections of several uninoculated samples became colonized with both *S. chartarum* and *Chaetomium globosum* (Table 1). These species were presumably part of the inherent bioburden on the gypsum wallboard following manufacturing and storage.

Stachybotrys chartarum, when inoculated onto the finish paper and kraft paper sides of used gypsum dry wall, produced nearly confluent colonizations after 14–28 days of exposure in moisture chambers at >95% RH (Fig. 2). The moisture content of the facing of the dry wall typically increased from 7–9% to near 35% on the finished side and to about 30% on the kraft side after 3 weeks at 95% relative humidity. A white hyphal mat was observed by days 7–10 with subsequent darkening as conidiogenesis progressed. A dark melaninated rinsate was obtained from the brushing of the colonized paper surface, but the paper, after cleaning and sanitization, appeared clean visually. With light microscopy (tape mounts) scattered conidia and hyphal fragments of *S. chartarum* were observed on the cleaned surfaces. In contrast, the paper facings of dry wall held at 85–88% relative humidity usually increased in water content to about 25% (finished) and 19% (kraft). Colonization by *S. chartarum* at these lower humidities at 3 weeks was less evident visually, but conidiogenesis on hyphae was observed microscopically. Ambient relative humidities at the lower and higher levels in the flasks tended to drift to near 90% with time and extent of fungal development.

In preliminary screening, all the sanitization solutions used at the manufacturer's recommended dilutions inactivated 10^6 conidia/ml of *S. chartarum* in 15 min, that is, viable colonies were not recoverable on neutralization, malt extract, or cellulose agars. Moreover, no fungi were recovered from the rinsates of the sanitizing solutions.

After the wallboard sections colonized with *S. chartarum* were cleaned and sanitized, they were held in the moisture chambers.

Visually observable fungal growth developed on the cross-sectional, non-coated edges of some of the sanitized and preserved dry wall samples after 14 days' incubation at >95% RH (Fig. 3). By day 21 in the humidity chamber, pinpoint fungal colonies were observed penetrating the clear, acrylic-based, cooling coil coating. Fungi were not observable on the metal primer paint until about 35 days. *Chaetomium globosum* and *Aspergillus flaviceps* and *A. ustus* were identified from tape mounts of the uncoated edges and in subsequent culture, whereas the areas coated with the metal primer containing the amine-neutralized phosphoric acid preservative remained free from recolonization for an additional 3 weeks. An unusual observation on these treated surfaces from one set of wallboard samples was the late formation of numerous small (<5.0 mm in diam.), orange, pasty colonies. These were found to present masses of hülle cells of *A. ustus*, presumably formed because of stress conditions (Fig. 4). During the first 7 weeks, *S. chartarum* was recovered only from control sections and sections sanitized by the quaternary mixture alone (Table 1).

Two sections that were disinfected with the quaternary and treated with the acrylic were transferred to a second challenge chamber after 40 days. These sections remained free of *S. chartarum* for an additional 90 days, whereas two sections treated with quaternary plus chlorine dioxide and the acrylic also appeared clean but became colonized with *Acremonium strictum* and *Ascotrichum lusitanica* (Fig. 5). Untreated controls were overgrown with *Chaetomium* spp.

The percentage moisture of the kraft paper of the primer-painted gypsum dry wall sections (n = 8), after 5 weeks in a moisture chamber, ranged from 31% to 34%.

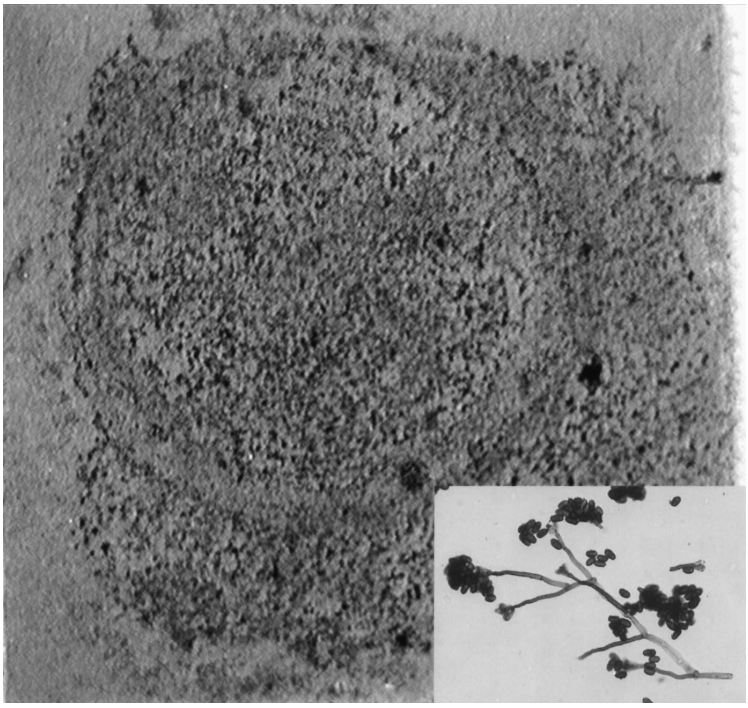

Fig. 2. Growth of *Stachybotrys chartarum* on celluose facing of gypsum wallboard at 95% RH at 22–24°C within 18 days (top); conidiophore with conidia (bottom).

The finished-paper side of the coated-gypsum wallboard (primer and acrylic, n = 18) had moisture contents of 37–42%. Control gypsum wallboard sections, which were densely colonized on two occasions, had moisture readings of nearly 50% on the finish-paper side and 37% on the kraft-paper side. Dry wall moisture content of the wall surfaces in the laboratory and in various residences in the area, and of uninoculated control sections (n = 35) were 8–12%.

Discussion

Total submersion or complete saturation of the dry wall substrate with water was not necessary for *S. chartarum* to grow and produce conidia. Preliminary data indicated that a water content near 30% was sufficient for dense colonization, but conidial germination and sparse growth was observed at about 25% moisture. During the past several years we have encountered colonizations of wallboard and ceiling tiles in at least 15 buildings in the southeastern United States. Most of these colonizations have been associated with direct water damage: burst pipes, roof leaks, floods, etc., but several extensive colonizations at three sites were at vapor barriers with marked temperature variances as the apparent cause of water accumulation. The moisture chambers provided for a simple assessment of the relative water activity that supported the growth of fungi on wallboard.

In laboratory challenge tests, conidia of *S. chartarum* were susceptible to 15 min of exposure to several concentrations of BIO_2, to 0.36% didecyl dimethyl

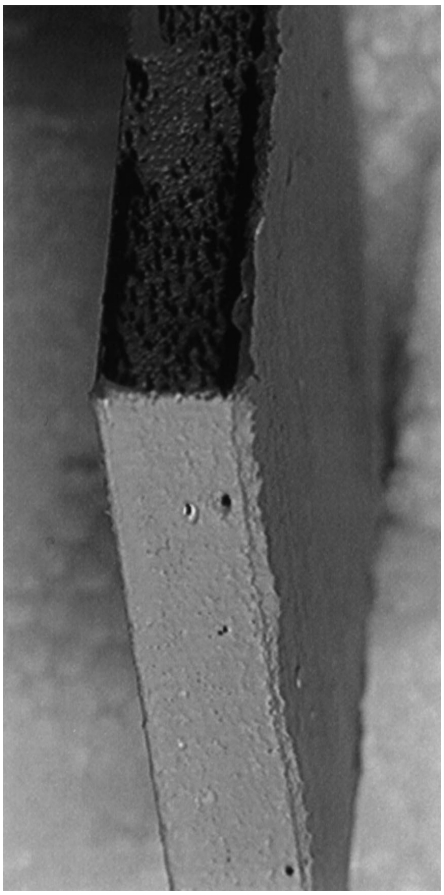

Fig. 3. Fungal development on non-treated edges of cleaned and quaternary-$C10_2$ sanitized wallboard after 14 days. *Penicillium* and *Chaetomium* spp. were isolated and identified on the surface by direct microscopy.

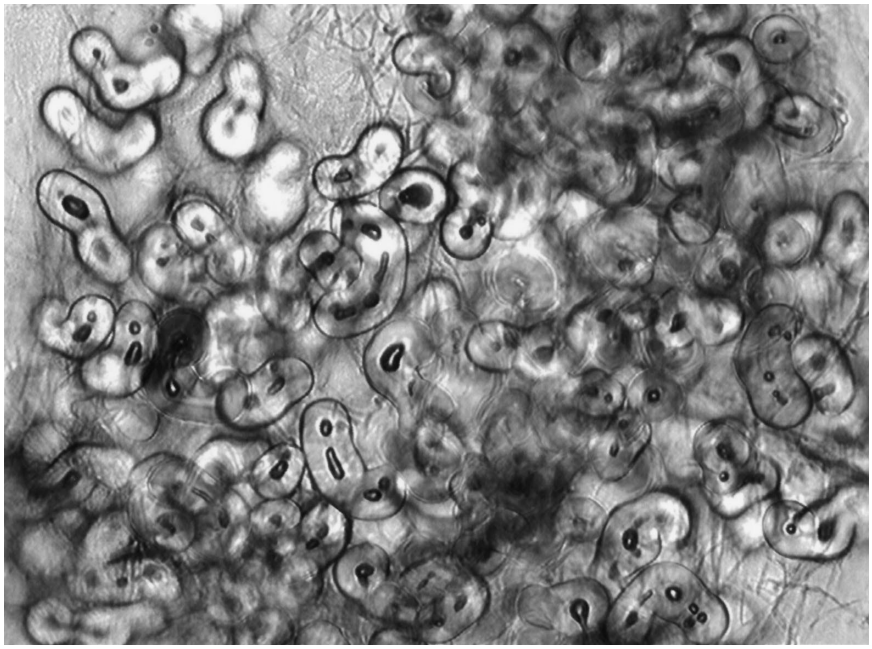

Fig. 4. Hülle cell colonies of *Aspergillus ustus* on preservative-treated wallboard.

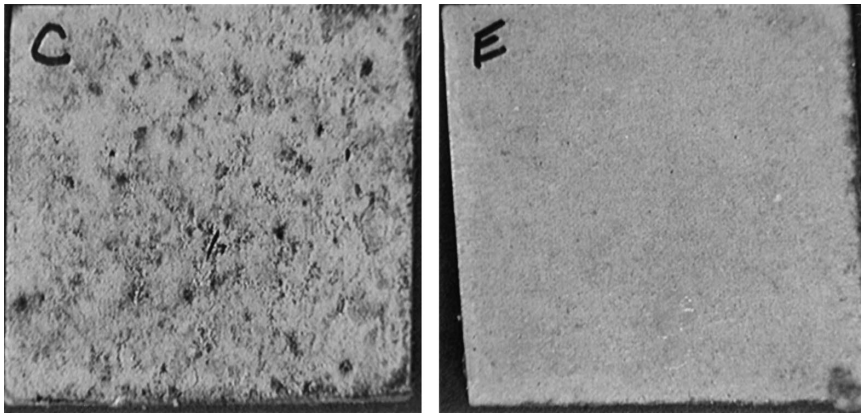

Fig. 5. Fungal development on untreated wallboard (left) versus development on quaternary-$C10_2$ sanitized and treated wallboard after 55 days at 90% + RH (right).

ammonium chloride and 0.023% n-alkyl dimethyl benzyl ammonium chloride, and to 0.72% chlorine dioxide combined with 0.4% didecyl dimethyl ammonium chloride, that is, no growth was recovered in Letheen neutralizing broth or agar nor on the enrichment agars. The inactivation of the conidia and mycelium of *S. chartarum* by these sanitizers also occurred on the dry wall, but in repeat tests other fungi and *Bacillus* spp. were recovered. BIO_2 and $Cl0_2$ were rapidly neutralized after application to wallboard and, without an added preservative step, regrowth eventually occurred. The BIO_2 sanitizer, which was nontoxic as used at a concentration of about 8 mg/L peroxidase activity, was of particular interest as it produced only a fleeting odor.

Samples sanitized and then treated with coatings containing the amine-neutralized phosphoric acid esters supported the growth of fungi other than *S. chartarum* after 14 to >35 days in vessels with initially high relative humidities (95–97%). The species that developed mainly, *Penicillium* and *Chaetomium* spp. in controls and *Aspergillus* and *Chaetomium* spp. on treated samples, were not used as inocula in the original challenges. The initial observations of the sites of growth on the cut edges suggested viable propagules of different species for different wallboards were embedded in the paper matrix at least for some of the sample studies. Because *S. chartarum* seems to be poorly competitive with other fungi [7] and because of its apparent sensitivity to sanitizers, sanitation and preservative treatment of wallboard that is still structurally sound but colonized by *S. chartarum* may provide a practical and safe remediative alternative for replacement of the wallboard. Additional isolates of *S. chartarum* grown on wallboard need to be examined for their susceptibility to sanitizers, and field trials on sanitation of infested wallboards need to be undertaken.

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