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Fungal colonization of fiberglass insulation in the air distribution system of a multi-story office building: VOC production and possible relationship to a sick building syndrome

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Complaints characteristic of those for sick building syndrome prompted mycological investigations of a modern multi-story office building on the Gulf coast in the Southeastern United States (Houston-Galveston area). The air handling units and fiberglass duct liner of the heating, ventilating and air conditioning system of the building, without a history of catastrophic or chronic water damage, demonstrated extensive colonization with *Penicillium* spp and *Cladosporium herbarum*. Although dense fungal growth was observed on surfaces within the heating-cooling system, most air samples yielded fewer than 200 CFU m^{-3}. Several volatile compounds found in the building air were released also from colonized fiberglass. Removal of colonized insulation from the floor receiving the majority of complaints of mouldy air and continuous operation of the units supplying this floor resulted in a reduction in the number of complaints.

Keywords: fungal colonization; fiberglass insulation; *Penicillium* spp; *Cladosporium* spp

Introduction

Complaints such as eye irritation, headaches and chronic fatigue have been related to working in large, nearly-sealed buildings [4,5]. These varied complaints, when in sufficient numbers (about 20% of the buildings' inhabitants), have been attributed to the 'sick building syndrome' (SBS). Various chemicals, such as those produced from electronic office equipment or from off-gassing of indoor construction and finishing materials, are often considered the cause of SBS, but microorganisms, particularly fungi, have also been implicated [9,11,17]. Wood, paper, textiles and similar materials are known substrates for indoor fungal growth that may produce airborne organics including mycotoxins [3,10]. In addition, metal surfaces [2,13], filter media [16], and thermal-acoustic fiberglass insulations in heating, ventilating and air-conditioning systems (HVAC) may be colonized by fungi [1,12,15]. In laboratory studies, rates of fungal colonization of the acrylic-latex facing of the fiberglass appeared related to the water content and adsorbed organics [6,14]. The matrices of the fiberglass were also found to be colonized, but colonization here seemed to be, in part, controlled by resistance to formaldehyde residues [7,8]. Fungal proliferation and growth in indoor settings is associated frequently with catastrophic or chronic water damage [12]. This study examines fungal populations that developed within 2 years in the HVAC system of a newly constructed large modern office building without a history of water damage.

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Materials and methods

Study site

A six-floor office building (near the Gulf coast of the Southeastern United States, Houston-Galveston area), equipped with computer-regulated heating and air conditioning, had been occupied for about 18 months prior to complaints from occupants of mouldy air. The building contained about 15 000 m^2 of work space and housed about 1000 workers. Each floor was serviced by two air handling units and the air was distributed through ducts lined with acoustic-thermal fiberglass. During the period of study (August 1994–August 1995) the recorded temperatures for the building ranged from 21–25°C; and the ambient relative humidities ranged from about 37% to 50%. Complaints of mouldy air were most numerous from occupants of the third floor. Shortly after the first series of complaints in August 1994, inspections involving preliminary cultures and microscopy of samples of insulation, indicated that the insulation in the air handling units of the third floor and the facing of the fiberglass duct liner from the third floor were moist and were colonized by fungi. The fiberglass insulation was removed from all the air handling units on all floors and from about the first 15 m of duct downstream of the fan on the third floor. The air handling units and abated duct were then cleaned with a NaOCl solution. After this process, additional samples were collected in mid-February and June 1995 and the samples were examined for fungi.

Samples

Air samples (1 min) were collected with a single-stage and on one occasion with a six-stage bioaerosol sampler (Graseby Andersen, Inc, Atlanta GA, USA). Fiberglass

insulation was collected from the air handling units on all six floors and placed in clean plastic bags. Most samples were from the first and third floors. Portals were cut into the metal ductworks, and fiberglass was collected downstream from the fans. These portals were equipped with doors and all sites were sampled at least three times over a ten-month period. At the second sampling, small sections of fiberglass, approximately 8 cm^2, were cut from the duct liners at selected portals and the sections were replaced with unused fiberglass of the same type. The fiberglass sections were placed in sterile bags for transport to the laboratory. We also used a modification of the pressure tape method to collect multiple samples from surfaces in the building. Clear polyvinyl adhesive tape, adhesive side down, was pressed directly onto the surface of suspect material. Five to seven impressions from the acrylic facing were made within 0.5 m of each bulk sample of fiberglass removed. The adhesive tape with attached surface materials was placed lightly, adhesive side down, on a labeled microscope slide. Only one end (10–15 mm) of the tape was pressed firmly to the slide. At the other end, 10–15 mm was folded (adhesive to adhesive) onto itself. The slides were placed in sealable plastic specimen slide holders and transported to the laboratory. In the laboratory, the tape was lifted and a drop of lacto-phenol cotton blue stain or a clear fixative was applied to the glass. The tape was then pressed over the fixative preparation and the slide was examined under a microscope. Surfaces of the fiberglass facing both at the building site and when the materials were returned to the laboratory were swabbed and streaked on isolation agars. Small sections of the fiberglass were imprinted also onto isolation agars.

Media

Malt Extract Agar, Mycological Agar and Czapek's Agar (Difco, Detroit, MI, USA) with 40% sucrose were employed routinely for the isolation of fungi from the air samples. All isolation agars were incubated at 22–26°C for up to 14 days. Pure cultures of representative colonies from isolation agars, including swab and bulk cultures of all fiberglass samples and water samples from the air handling units, were identified by standard morphological criteria.

Volatile organic compounds (VOCs)

VOCs from fiberglass were detected according to the procedures of Ezeonu *et al* [7]. Two to four 8-cm^2 sections of fiberglass duct liner from three separate collections from the third floor were placed into 100-ml borosilicate purge-and-trap vessels (Wheaton, Millville, NJ, USA). Approximately 0.5 ml of sterile distilled water was added to the fiberglass and the vessels were sealed for 10–21 days. Non-colonized fiberglass sections of the same type as that in the building and cultures of the dominant fungi isolated from the fiberglass were also analyzed for VOC production. Headspace air in the trap vessels was drawn through a desorption tube packed with Carbotrap C (3 cm), Carbotrap (3.5 cm) and Carbosieve (1.5 cm) sorbent charcoals (Suppelco Inc, Bellefonte, PA, USA). The charcoal bed containing the adsorbed organic compounds was purged with helium and thermally volatilized and cryofocused with a Chrompack TCT unit (Raritan, NJ, USA). The captured compounds were thermally desorbed (250°C) and injected into a Hewlett-Packard GC column. Detection was performed with a Hewlett-Packard 5971A mass selective detector in a scan mode with a range of 30–300 atomic mass units. Spectra were analyzed and peaks were identified with comparisons to the NIST/EPA/MSDC 49K Mass Spectral Database (US Department of Commerce, Washington, DC, USA). The detection limit for organics was 50 mg m^{-3}, based on toluene as the external standard. The identifications of certain compounds involved comparisons with additional external standards.

Scanning Electron Microscopy (SEM)

Sections of the fiberglass insulation surface layer, approximately 1 cm^2, were removed from representative bulk samples with a clean razor blade. These sections were mounted on aluminum SEM specimen stubs and sputter coated with approximately 15 nm of gold/palladium in a Denton Desk II coating unit (Denton Vacuum, Morrestown, NJ, USA). The specimens were examined in a Leica S420 scanning electron microscope (Leica Cambridge, Ltd, Cambridge, UK) operating at 5 kV. Images were recorded digitally and stored as TIFF files.

Results

The fungi identified from the building from 12 air samples and direct sampling of surfaces are listed in Table 1. One-minute air samples plated on the various media never yielded more than 25 colonies per plate and usually yielded fewer than six colonies. In contrast, swab samples of most surfaces associated with the air handling units and thermal acoustic fiberglass yielded confluent growth (Table 2). *Penicillium* spp, tentatively identified as *P. simplicissimum–P. janthinellum* (series) and *P. chrysogenum*, were prominent in the August 1994 samples. Visually detectable fungal growth was obvious on the fiberglass facing of initially collected samples (Figure 1). Multiple microscopic preparations (at least seven from each section of fiberglass) from the third floor exhibited a uniform coating with fungi

Table 1 Fungi identified from a multi-story office building

Species	Floor	Source[a]	Range of CFU m^{-3} air[b]
Alternaria alternata	5,6	air	<1–70
Aspergillus fumigatus	1,6	air	<1–35
Aspergillus flavus	5	air	<1–35
Aspergillus niger	1,3,5	air, ahu	<1–105
Cladosporium cladosporioides	1–6	air, ins, met	165–420
Cladosporium herbarum	1–6	air, ins, ahu, met	165–420
Curvularia lunata	3	ins, air	<1–70
Epicoccum nigrum	3	air	<1–35
Geotrichum candidum	2	air	<1–35
Penicillium spp	1–6	ins, air	<1–300
Rhizopus stolonifera	6	ins, air	<70–165

[a]Observed microscopically and recovered in culture: ahu, air handling unit; ins, facing of fiberglass duct insulation; met, metal housing of units or fans.
[b]Estimated from CFU on isolation agar that gave highest number from 1 min with Andersen air sampler ($n = 3$–5).

Table 2 Principal fungi colonizing fiberglass insulation and fungi in air in a multi-story office building

Collection dates	No. fiberglass samples colonized/No. samples examined	Principal fungi found	Maximal density in air m^{-3}
Aug–Sept 1994	10/10	*Cladosporium herbarum* *Penicillium* spp	682
Feb 1995	79/85	*Cladosporium herbarum* *Penicillium* spp	300
June 1995	13/13[a]	*Cladosporium herbarum,* *Penicillium janthinellum,* *Paecilomyces lilacinus*[b]	ND[c]

[a]Samples from first three floors, samples from all floors on other collection dates.
[b]Isolated from all samples, but microscopic evidence of colonization not obtained.
[c]Not done.

(Figure 2a, b). Colonization of the fiberglass with the *Penicillium* spp appeared heaviest closer (first 20 m of duct work) to the air handling units. *Cladosporium herbarum* was most evident in samples collected in February and June 1995. In February 1995, *Paecilomyces lilacinus* was isolated from standing water in the bottom of the air handlers from the third floor. These air handlers were being treated with a quaternary biocide. All fiberglass samples examined, except for six subsamples collected from a distant section (75 m) of the duct on the third floor, were colonized densely with fungi. In some samples, hyphae penetrated into the fiberglass matrix (Figure 2c). Fungal colonization on fiberglass from the first floor occurred in discrete patches and conidia production appeared limited (Figure 2d). *Streptomyces* sp and a *Bacillus* sp that inhibited fungi on isolation agar were obtained from these latter samples.

In June 1995, dense growth of *P. lilacinus* was recovered from swab culture of the insulation in the third floor duct more than 24 m downstream from the fan. There was evidence of active growth of *Penicillium janthinellum* on the insulation facing (mature conidiophores and conidia arising from vegetative hyphae), but no definitive sporulating structures of *Paecilomyces lilacinus* were observed. New sections of fiberglass insulation inserted into the ducts during the February 1995 sampling were colonized by fungi, particularly *C. herbarum*, when they were examined in June 1995 (Figure 3a, b). Air samples did not reflect the high densities of fungi observed on the fiberglass insulation and the air yielded a greater species diversity, including occasional cultures of *Aspergillus fumigatus, Geotrichum candidum* and *Rhizopus* sp. Fungal colonization of other surfaces in the HVAC system (metal fans, vents, etc) was not observed.

The abatement procedure, undertaken in late summer 1994, was followed by a decrease in the perception of the mouldy odor. In November 1994, a preliminary screening of volatile organic compounds (VOCs) in the air in representative rooms on the first and third floors was conducted by an outside laboratory. More than 30 VOCs were detected in the air with total concentrations on the first floor ranging to 360 $\mu g\ m^{-3}$ and up to 1500 $\mu g\ m^{-3}$ on the third floor. Toluene and methylene chloride were prominent compounds, and benzene derivatives and trichlor- and tetrachlor-ethanes and ethenes were present (K Strawn, Houston, personal communication). VOCs detected in emissions from colonized insulation collected in February 1995 that were not released from noncolonized control insulation are indicated in Table 3. More than 30 VOCs were emitted from the insulation samples including methylene chloride, tri- and tetra-chlor ethanes and ethenes and benzene derivatives. These same compounds were reported earlier in the air. The colonized material gave off a geosmin-like odor but geosmin was not indicated in the VOC

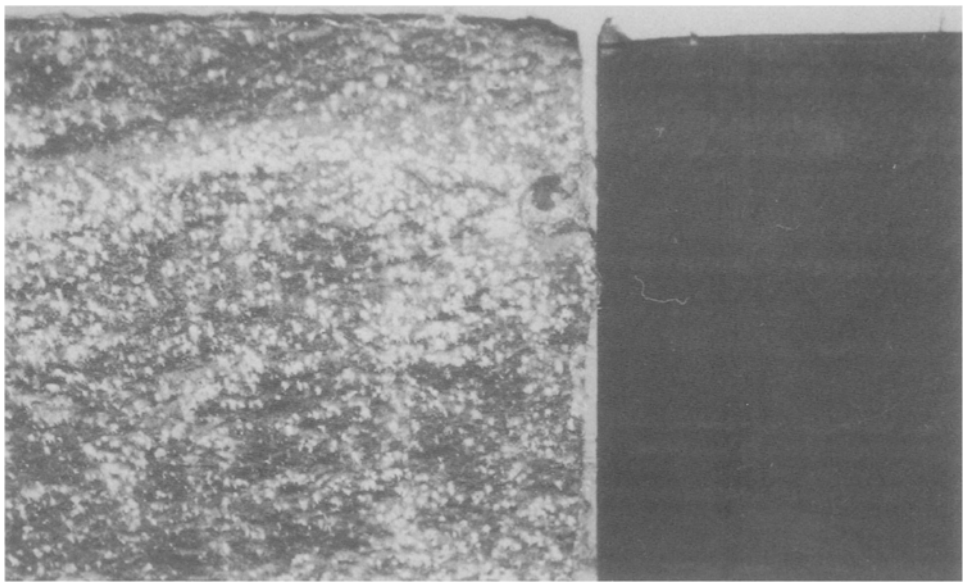

Figure 1 Fungal colonization of facing of fiberglass insulation in place in third floor ducts of a multi-story building for 18 months (Left) as compared to unused insulation (Right) of the same type.

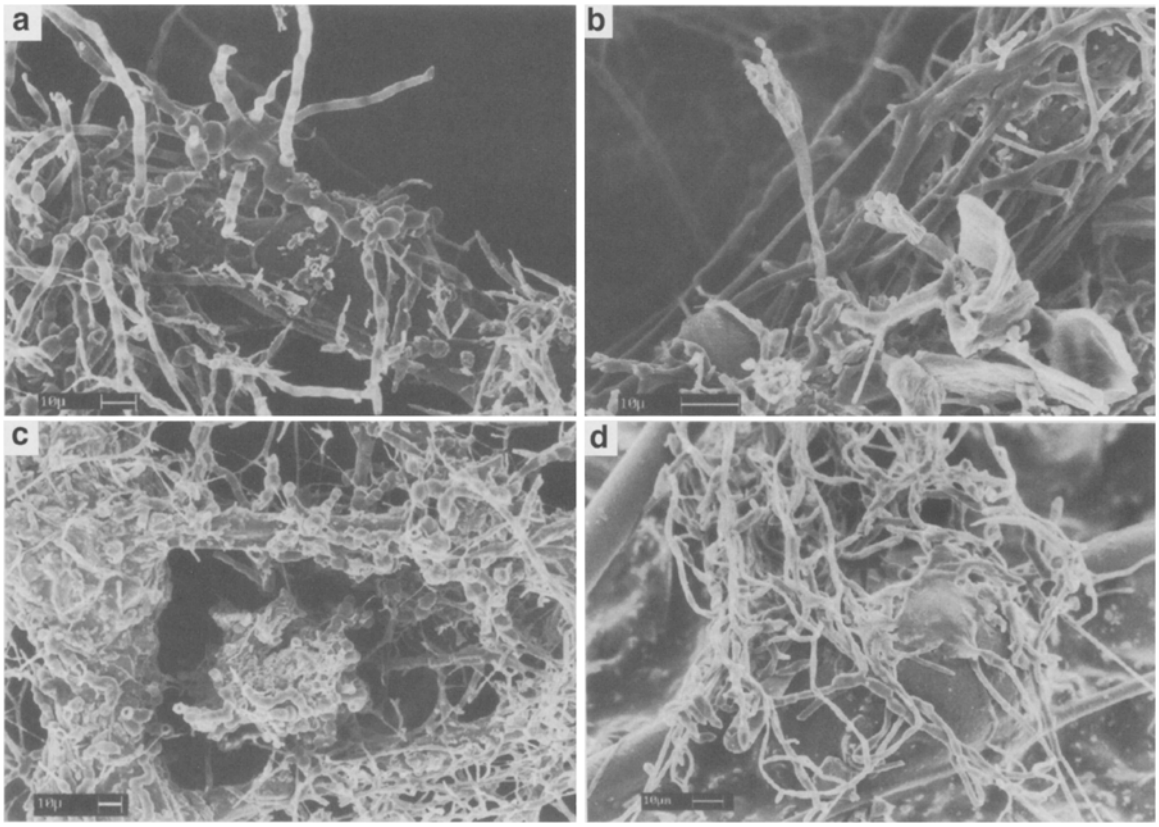

Figure 2 Fungal colonization of representative surfaces of thermal-acoustic fiberglass insulation removed from HVAC ducts of a multi-story office building after 18 months. (a) Area of acrylic-latex facing that yielded *Cladosporium* sp upon culture. (b) Area of acrylic-latex facing that yielded *Penicillium* sp and *Cladosporium* sp upon culture. (c) Hyphal penetration of matrix of fiberglass (sections a, b, and c from third floor). (d) Hyphal colonization of acrylic-latex facing of insulation from first floor. Scale bars = 10 μm.

Table 3 VOCs of possible fungal origin emitted from colonized fiberglass insulation[a]

VOC	Percent of total VOCs
Hexanal	15.8
2-Cyclohexen-1-o1	2.1
2,3,4-Trimethyl hexane	1.3
2-Butyl-1-octanal	1.3
2-Pentenal	0.15

[a]Compounds (not observed in noncolonized control samples) have library confidence values above 60% for gas chromatography-mass spectrometry identifications.

analyses. A comparison of organic compounds released from colonized insulation collected from the third floor in June 1995 and from a mixed culture from the insulation is presented in Table 4. The data were obtained from samples processed separately in time and space from the aforementioned air samples. Agar blanks did yield some of the same VOCs detected in the culture systems but the concentrations varied. Ethyl hexanol was indicated for the culture system, but not the colonized fiberglass. Previously, ethyl hexanol, a known eye irritant, was shown to be produced directly from colonized fiberglass as well as in enrichment culture [7].

Discussion

In an earlier study, we reported on the colonization of relatively new fiberglass duct liner in the HVAC system of a large single residence [1]. The duct liner in this residence (Type A, authors' designation) was found in a later study to be relatively more susceptible to fungal colonization than another duct liner (Type B) [6]. The type B duct liner studied by Ezeonu *et al* [6] appeared to be the same type duct liner colonized in the multi-story building of this study. *In situ* colonization of the type B duct liner in the multi-story building was observed to about the same extent and within the same time as reported for Type A duct liner in the single residence [1]. The fungi at both sites were similar types, but species varied and colonizing aspergilli were not observed in the office building. At both sites, periodic blooms of *Penicillium* spp occurred with a more or less constant establishment of *Cladosporium* sp. The mouldy odors appeared (based on culture observations) to be associated with the prevalence of the *Penicillium* spp or the *Aspergillus* sp. At both sites, antagonistic relationships were noted between species from different sites within the HVAC systems and antifungal *Streptomyces* spp that produced geosmin in culture were observed. Geosmin-like odors were produced also from the fungal cultures from both sites but geosmin was not detected from the fungi in the GC-MS analyses.

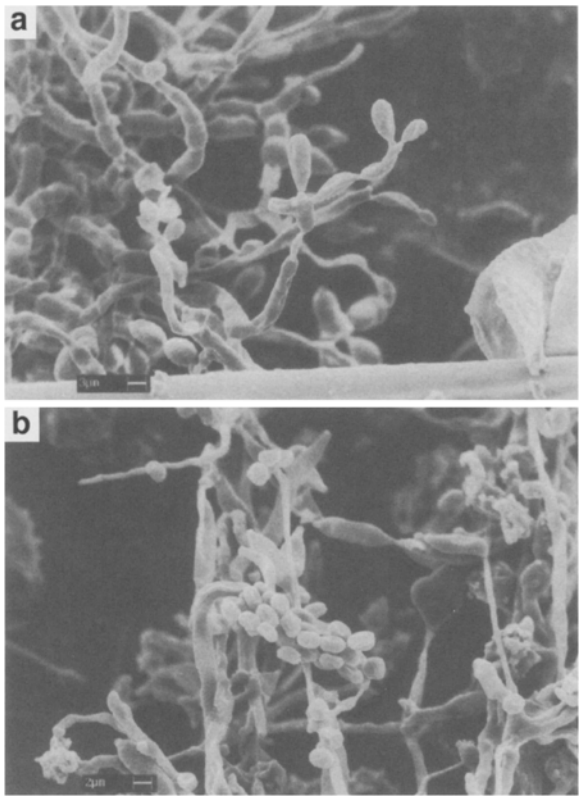

Figure 3 Fungal colonization of acrylic facing of fiberglass insulation after 90 days of use. (a) *Cladosporium* sp, scale bar = 3 μm. (b) *Cladosporium* sp and *Penicillium* sp, scale bar = 2 μm.

The association of conidia-release and volatile production with complaints and symptoms of occupants is problematic. Our analyses for volatiles along with our earlier studies [3,7] and the fungal-like odors in the building suggest that fungi contribute to the total VOCs and the complaints of the occupants. Our data, however, are restricted in time and were collected under non-dynamic environmental conditions. After incubation in the trap flasks, colonized insulation often yielded a lesser number of volatiles than noncolonized insulation, and noncolonized 'controls' yielded highly variable results probably reflecting their storage. Obviously, more intense studies of volatile production by fungi are necessary.

A significant indoor ecological phenomenon is the effect of cycling of air handling systems on the humidity and water activity within HVAC systems. Many commercial buildings and schools are operated on an 'economizer cycle' where the airflow is reduced or the system is completely shut down during off-peak or non-office hours, ie evenings and/or weekends. During these periods water activity can increase on surfaces due to rising temperatures and humidity. The complaints of musty or mouldy air, as in the building studied here, are generally more numerous on Monday mornings or during periods immediately following activation of the HVAC system, particularly to full cooling and dehumidifying capacity. In our experience, modern buildings operating on energy-efficient cycles in humid regions of the country have more microbe-related problems than those with units operating continuously. In this regard, after the units for the first and third floors were kept in continuous operation, in the latter phases of this study, complaints of objectionable air decreased and the

Table 4 Volatile organic compounds released from colonized insulation from the multi-story building and from a mixed culture isolated from the insulation

Colonized insulation		Culture from insulation	
Volatile compounds identified	Percentage	Volatile compounds identified	Percentage
Methane, chloro-	0.45[b]	Methane, chlorofluoro-	1.64
Ethane, 1,1,2-trichloro-1,2,2-trifluoro-	2.38	Methane, chloro-	0.82
Hexane	2.69	Trichloromonofluoromethane	3.46
Chloroform	1.05	Ethane, 1,1,2-trichloro-1,2,2-trifluoro-[a]	0.98
Ethane, 1,2,2,-trichloro-[a]	0.58	Methylene chloride[a]	0.75
Benzene	1.95	Silanol, trimethyl-	2.44
Toluene	1.74	1-Pentene, 2-methyl-	0.91
Tetrachloroethylene	5.53	Hexane[a]	4.85
1-Heptene, 2,4,-dimethyl-	1.28	Benzene	4.51
1-Pentene, 2-methyl[a]	1.27	Triethylamine	4.37
1-Octene	1.72	1-Cyclohexane, 1-methanol[a]	1.98
1-Heptanol, 6-methyl-	17.8	Toluene	2.28
Isooctanol[a]	36.5	Tetrachloroethylene	10.5
1-Dodecene	1.31	2-Pentene[a]	2.82
Dodecane	3.99	Octane, 4-methyl-	1.79
		1-Hexanol, 2-ethyl-	4.37
		Decane, 2,4-dimethyl-	6.34
		6-Dodecene, (E)[a]	3.21
		Decane, 3-methyl-	9.92
		Cyclohexasiloxane, dodecamethyl	3.39
		Eicosane, 2,6,10,14-tetramethyl-	2.31

[a]The identified compounds except those with an ([a]) have library confidence values of above 60%.
[b]Relative percent of total volatiles.

microscopic and culture studies demonstrated that the colonizing fungi had shifted from *Penicillium* spp mainly to *Cladosporium* spp. The air-handling units in this building during all of our observations held reservoirs of water. Fan activation most probably facilitated the passage of moisture down the ducts.

Available moisture as well as the susceptibility of the particular type of fiberglass and its absorption of VOCs mediate the extent of fungal growth. If the fiberglass substrate itself does not readily support growth, then the level and type of total organics appear to stimulate or inhibit selected fungi [7]. Accumulation of organic debris (eg pollen) which also may support fungal growth in the duct work may occur rapidly in certain environments (pollen was observed in the ducts in this study). Possibly, fungal colonization may go undetected because the primary colonizer does not produce perceptible odors. Colonization of fiberglass with objectionable fungi in one of several similar buildings with the same environmental conditions is probably a matter of chance. The interactions of the primary colonizer(s) and subsequent community with the complex dynamics of the energy-efficient HVAC system require further study. Proper engineering design, scrupulous maintenance, appropriate filtration media and strict control of moisture are obvious necessities for controlling fungi in HVAC systems.

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