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Colonization of fiberglass insulation used in heating, ventilation and air conditioning systems

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SUMMARY

The number of fungal species colonizing thermal and acoustic fiberglass insulations used in heating, ventilation, and air conditioning (HVAC) systems was fewer than that obtained from initial direct culture of these insulations. The colonization, determined by the microscopic observation of conidiophores with conidia, was primarily of acrylic-latex-facing material, but eventually the fungi permeated the fiberglass matrix. Isolates of *Aspergillus versicolor* were most often obtained from non-challenged insulation, whereas *Acremonium obclavatum* appeared to be the primary colonizing fungus in high-humidity (>90%) challenge chambers. At a lower humidity (about 70%) *Aspergillus flavus* was one of the more prominent fungi. Not all duct liner samples were equally susceptible to colonization and duct board appeared relatively resistant to colonization.

INTRODUCTION

There have been several reports of fungal colonization of dirty and moisture-laden fiberglass insulation associated with heating, ventilating and air conditioning (HVAC) systems [3,4,5]. We have observed in situ fungal growth with development of mature reproductive structures in HVAC systems in association with painted metal air vents [2], filters [10] and the acrylic latex facing of duct liner [1]. Colonization of duct liner occurred in relatively clean and well maintained HVAC systems and some new insulation materials were colonized readily [1]. The extent of colonization into the fiberglass matrix was not determined. This study examines the recovery of fungi from unused fiberglass insulation and the susceptibility of various duct liners and duct board to fungal colonization.

MATERIALS AND METHODS

Samples of fiberglass duct liners and duct board were placed in an environmental challenge chamber (29–34 °C, relative humidity >90%) which contained over 50 species of fungi. The chamber is described in detail elsewhere [1,8]. Various non-insulation indoor construction and finishing materials were introduced periodically into the environmental chamber during the study. Air sampling of the chamber revealed conidia levels greater than 14000 colony-forming units per cubic meter of air. In addition, two modified environmental challenge chambers without fans or heaters (which were present in the above chamber), were kept at 22–25 °C with relative humidities of 65–70% and >90%. These were used for challenge of the fiberglass insulations only. Three different types of fiberglass insulation, two brands of duct liner and a more rigid duct board, were purchased retail, cut into 4–8 by 10–12 cm sections, and multiple samples were positioned on stainless steel racks or suspended with stainless steel wire within the environmental challenge chambers. The insulation sections were maintained in the chamber from 28 to 270 days. For controls, sections of new duct liners and rigid fiberglass duct board were placed in sterile petri dishes and incubated in separate non-challenge chambers with a temperature of 25–27 °C and relative humidities of 65–70% and 90%. In separate experiments, the acrylic-facing material on sections of some materials in all chambers were coated with an additional layer of an acrylic-latex. Half of the coatings contained an antimicrobial quaternary-phosphated amine complex [9].

Periodically, representative sections were removed from the chamber and examined microscopically for fungi. Samples were examined by direct epi-illumination light microscopy. Acetate tape mounts were taken from the surface for examination under transmitted light microscopy with Nomarski DCI Optics (Olympus Corp., Lake Success, NY) [1]. Colonization was assessed both microscopically and macroscopically. Microscopic colonization was based on observations of the development of conidiophores with mature conidia or other reproductive structures. Macroscopic colonization was based on the observation with the unaided eye of fungal mycelium with or without reproductive structures. Specimens for scanning electron microscopy (SEM) were mounted directly onto SEM stubs, sputter coated with Au/Pd, and examined in a JEOL JSM 35CF

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(JEOL, Inc., Peabody, MA) scanning electron microscope. All materials, including sections of new insulation that were not challenged in the chambers, were cultured on various enriched agars. Agars used included Mycological Agar (Difco, Detroit, MI) with 0.5 g L^{-1} chloramphenicol, as well as Czapek's Agar with sucrose or glycerol. Identifications of fungi were based on comparisons of reproductive structures from the insulations with those developed in pure culture. Identifications were based on standard morphological and physiological procedures.

RESULTS

A variety of fungi was isolated from unused fiberglass duct liners and duct board prior to their placement in the chambers (Table 1). Generally, most sections yielded only one or two colonies, but occasionally an entire section was enveloped in mycelium; this occurred with rapidly growing fungi such as *Monilia sitophila* and *Rhizopus stolonifer*. Representatives of *Aspergillus versicolor* group (*A. versicolor* and *A. sydowii*), were the most common isolates from unused insulation materials (Table 1, Fig. 1). Because the morphological distinction of these species is questionable, we will refer to this complex as *A. versicolor*. Microscopic examination of the fiberglass matrix and facing of unused materials revealed extensive fungal hyphae, on initial observation, only on two occasions. Not all fungal types observed microscopically were recovered in culture. The *A. versicolor* frequently required more than 10 days to produce colonies on mycological isolation agar (Fig. 2). *A. versicolor*, however, did colonize sections of controls in non-challenge chambers.

The incidences of fungi colonizing the various duct liners and duct board are indicated in Table 2. The different brands of materials varied in their susceptibility to colonization. All samples of duct liner A were colonized by fungi; macroscopic growth on the acrylic facing was observed in the challenge chamber on some samples within 40 days. Duct liner B showed macroscopic growth mostly on the cut ends of the fiberglass by 180 days. In the high humidity challenge chambers the primary colonization was by *Acremonium obclavatum* (Table 1), both on the facing material (including foil) and peripheral fiberglass filaments (Fig. 3). Rigid fiberglass duct board generally was more resistant to colonization, and only microscopic colonization was observed.

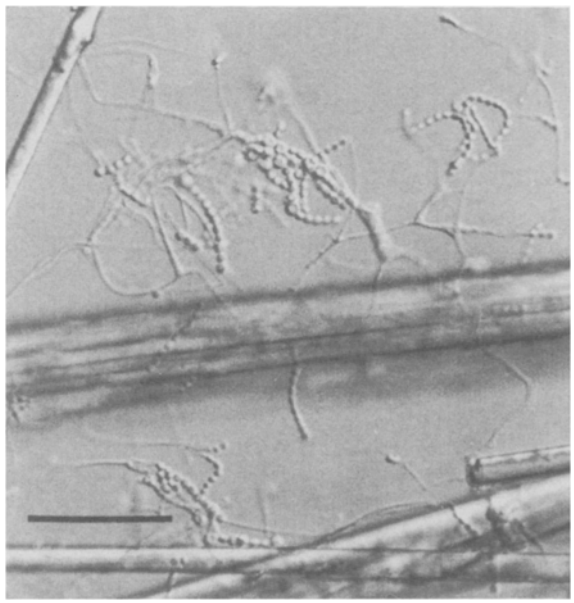

Fig. 1. Microscopic colonization by *Aspergillus versicolor* of fiberglass filaments of duct liner B from a non-challenge chamber. Bar = 50 μm.

Because fungal colonization of duct liner B was relatively slow, several sections that had been exposed in the environmental challenge chambers were examined for fungal penetration into the fiberglass matrix. An area about 1.0 cm^2 of the facing material was carefully removed from a section of the insulation and sequential layers of the underlying matrix (approximately 10 mm depth) were mounted on slides for microscopic observations. Sections also were inoculated

TABLE 1

Principle fungi colonizing fiberglass duct liners[a]

Initial culture (new insulation) (54)[b]	Challenge chamber (48)	Non-challenge control chamber (24)
Alternaria alternata (2)[c]	*Acremonium obclavatum* (48)	*A. versicolor* (3)
Aspergillus niger (3)	*Aspergillus flavus* (16)	*Chaetomium aureum* (8)[d]
A. versicolor (9)	*A. versicolor* (12)	*Cladosporium* spp. (8)[d]
Cladosporium spp. (7)	*A. niger* (7)	
Monilia sitophilia (2)	*Chaetomium* spp. (8)	
Penicillium spp. (6)	*Cladosporium* spp. (26)	
Rhizopus stolinifer (4)		

[a]More than one species possible on an individual sample.
[b](Total samples).
[c](Number positive of total samples).
[d]Observed mainly on facing material.

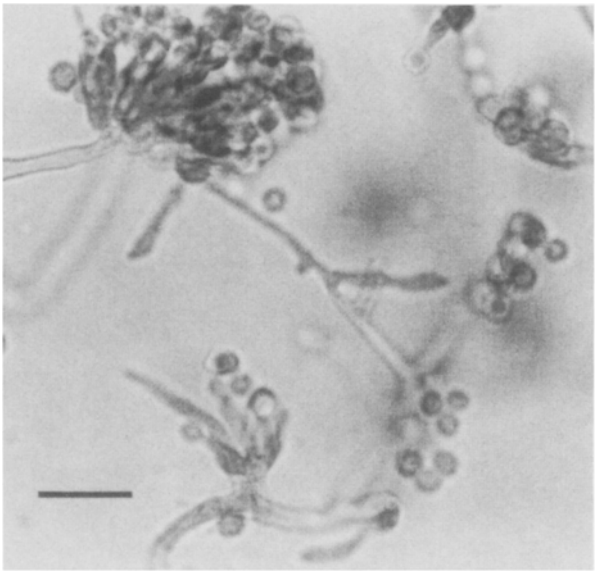

Fig. 2. Variation in conidiophores of *Aspergillus versicolor* in initial culture. Bar = 10 μm.

TABLE 2

Fungal colonization of fiberglass insulation

Sample	Days exposure in chamber		
	<30	30–90	90–270
Environmental challenge chambers			
Duct liner A	22/22[a]	22/22	22/22
Duct liner B	4/26	14/26	20/26
Duct board	0/14	1/14	9/14
Duct liner B[b]	2/13	3/13	6/13
Non-challenge chambers			
Duct liner A	0/8	1/8	8/8
Duct liner B	0/6	0/6	2/6
Duct board	0/8	0/8	nd[c]
Duct liner B[b]	0/2	0/2	2/2

[a]Number positive/number tested (2–8 cm^2 samples).
[b]Facing treated with a coating containing a phosphated quaternary amine complex.
[c]Not done.

onto enriched agars. Light microscopic examination of the layers showed concentrations of apparent conidia at each level in the matrix, but hyphal elements were sparse and conidiophores were not observed. Material placed onto mycological agar containing chloramphenicol yielded growth at all layers. The fiberglass matrix yielded mainly *Aspergillus versicolor* and *A. flavus* at lower humidities, and *Acremonium obclavatum* at the higher humidity. In the non-challenge chambers, microscopic colonization on duct liner A was mostly by *Cladosporium herbarum* and *Chaetomium aureum* (Fig. 4), whereas on duct liner B mainly *Aspergillus versicolor* was observed. *Aspergillus versicolor* also produced sparse colonization of three control samples. The microscopic colonization of duct liner B and duct board in the non-challenge chamber occurred only in isolated sectors.

None of the sections treated with the antimicrobial compound demonstrated macroscopic fungal growth on the facings over a 270-day exposure period. A few isolated conidiophores with conidia were observed microscopically on the facing of sections in the challenge chamber. The fungi, mainly *A. flavus* and *A. niger*, were inhibited by the antimicrobial in subsequent seeded agar overlay testing, indicating that the antimicrobial coating probably was not continuous. The colonization of the peripheral glass fibers was observed on treated sections regardless of processing (including challenge and non-challenge chambers).

DISCUSSION

Our study has shown that several new (unused) acoustic and thermal fiberglass duct liners and a type of duct board are subject differentially to colonization by fungi. In a high humidity chamber containing over 50 different fungi, only a few species colonized fiberglass duct liners and duct board. The primary colonizing species in the challenge chambers was *Acremonium obclavatum*. Occasionally in these chambers *Aspergillus flavus, A. versicolor, A. niger*, and *Chaetomium* spp. were the principal fungi on the acrylic-latex facings. In challenge chambers with lower humidities (65%–70%), *A. obclavatum* was not prevalent. Usually the acrylic-latex coatings appeared to be colonized first, but eventually, the fungi spread to the fiberglass matrix.

Fiberglass duct board seemed less susceptible to colonization by fungi. Sparse areas of colonization were usually observed along the foil facing on the outer edge and then, after 90 days, only on filaments projecting from the cut edges. The processing of rigid duct board may have an effect on its ability to resist fungal colonization. The duct board materials are much more compressed and may be processed at higher temperatures. Even the surface of the duct liner that is glued to the duct work seemed less susceptible to colonization than the cut sides. The colonizing species were not always the same as those isolated from the initial culture of the materials. *A. versicolor*, however, occurred at a relatively high incidence among non-challenged samples, and occasionally colonized insulation in all chambers.

The incorporation of a phosphated-amine antimicrobial treatment into the acrylic-latex facing of the duct liners reduced colonization even after extended exposure (270 days) in the environmental chamber. Similar results were noted in the non-challenge chambers. Colonization, however, was still noted on peripheral fibers protruding from the fiberglass matrix in the high humidity challenge chamber regardless of the presence of a treatment in the facing. Actual in-use conditions would tend to reduce the number of cross-sectional edges of the duct board and duct liner exposed to environmental conditions, however, the need to

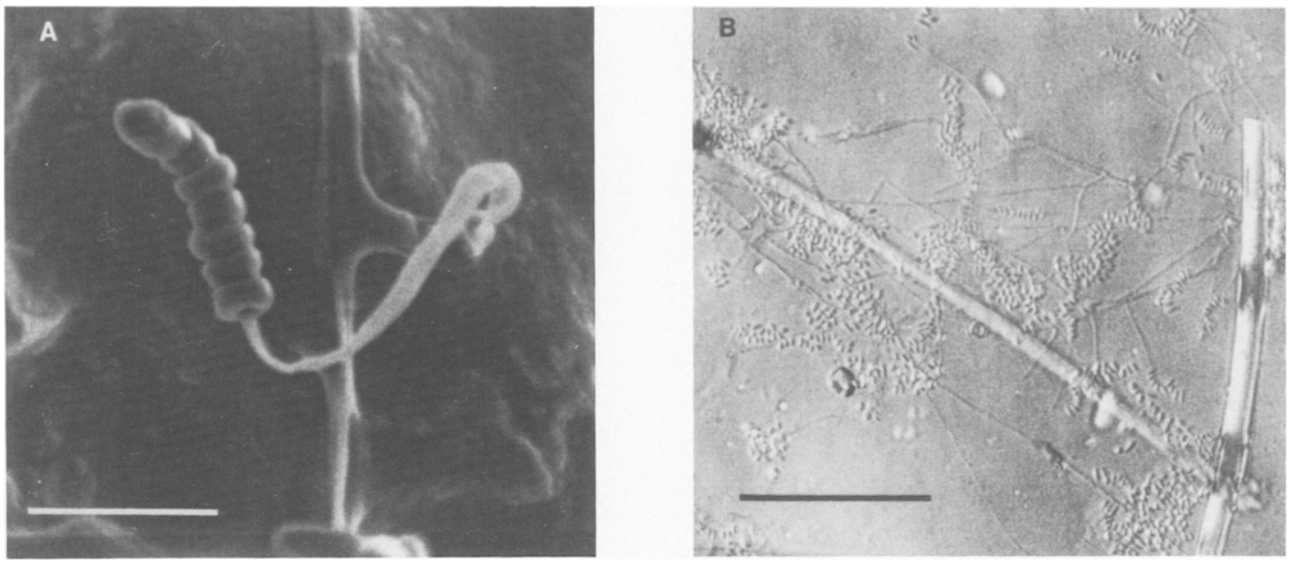

Fig. 3. Acrylic-latex facing of duct liner B with attached hyphae and conidiophore with conidia of *Acremonium obclavatum* from challenge chamber (SEM) (A) bar = 5 μm; *A. obclavatum* on filament of fiberglass from duct liner B in challenge chamber (B) bar = 50 μm.

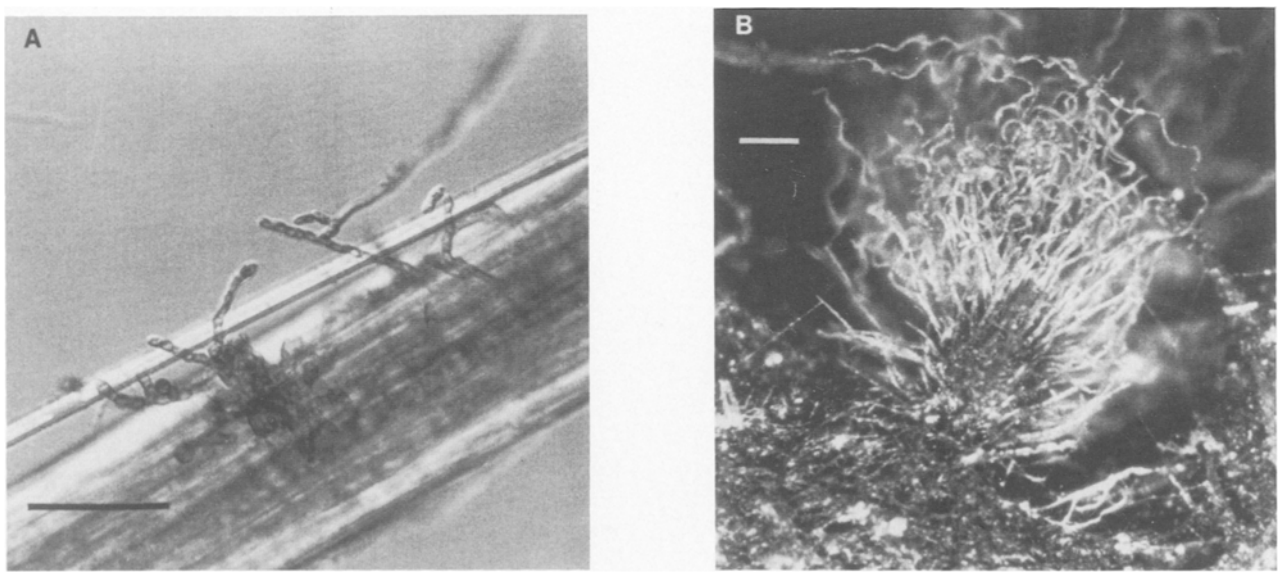

Fig. 4. *Cladosporium herbarum* associated with fiber glass filaments from duct liner A in a non-challenge chamber (A) bar = 50 μm; cleistothecium of *Chaetomium aureum* on acrylic-latex facing of duct liner A from a non-challenge chamber (B) bar = 100 μm.

provide a more recalcitrant face coating particularly for certain types of duct liner seems evident.

New fiberglass duct liner and rigid fiberglass duct board are reported to be unable to support fungal growth when tested with standard challenge procedures [6]. The evaluation criteria for such standard tests is usually based on comparative growth on the test substrate versus a cellulose substrate. If growth on the test substrate does not exceed the control cellulose substrate within 28–56 days, the sample is judged to pass the test. Growth in 28 days on fiberglass materials in our studies was sparse or observed only with microscopy. The latter observations also may be masked by the filamentous appearance of the fiberglass itself. Additionally, macroscopic growth was usually observed only after extended exposure to moisture and possibly volatile organics. Obviously in situ not all fiberglass insulations become contaminated by fungi, but contamination does occur [1,3,4,5]. Fiberglass itself should not support fungal growth, but the acrylic facings, and the mastic that maintains the physical integrity of the insulation material (duct liners and duct

boards) are suspect. Moreover fiber glass insulations have a hygroscopic nature and condensation in metal duct work itself does permit fungal growth under certain conditions [7,11].

Improved standard testing methods and detailed evaluation criteria should be developed for products with intended uses for many years, especially those in the interior of air handling units. Preservation of such products with low toxicity antimicrobials may be necessary for maintaining functional acoustic and thermal properties and for preventing potential adverse effects from colonizing fungi.

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REFERENCES

1 Ahearn, D.G., D.L. Price, R.B. Simmons and S.A. Crow Jr. 1992. Colonization studies of various HVAC insulation materials. In: IAQ '92 Environments for People, pp. 179–183, American Society of Heating Refrigeration and Air Conditioning Engineers, Inc., Atlanta, GA.

2 Ahearn, D.G., R.B. Simmons, K.F. Switzer, L. Ajello and D.L. Pierson. 1991. Colonization by *Cladosporium* spp. of painted metal surfaces associated with heating and air conditioning systems. J. Ind. Microbiol. 8: 277–283.

3 Morey, P.R. 1993. Microbiological contamination in buildings: precautions during remediation activities. In: Indoor Environment '93 Conference Proceedings, pp. 128–134, IAQ Publications, Bethesda, MD.

4 Morey, P.R., W.G. Jones, J.L. Clere and W.G. Sorenson. 1986. Studies on sources of airborne microorganisms and on indoor air quality in a large office building. In: IAQ '86: Managing Indoor Air for Health and Energy Conservation, pp. 500–507, American Society of Heating Refrigeration and Air Conditioning Engineers Inc., Atlanta, GA.

5 Morey, P.R. and C.M. Williams. 1991. Is porous insulation inside an HVAC system compatible with a healthy building? In: IAQ '91 Healthy Buildings, pp. 128–135, American Society of Heating Refrigeration and Air Conditioning Engineers Inc., Atlanta, GA.

6 North American Insulation Manufacturers Association. 1993. Cleaning Fibrous Glass Insulated Air Duct Systems; Recommended Practice. Publication No. A/H-122 3/93. Alexandria, VA.

7 Pasanen, P., A.-L. Pasanen and M. Jantunen. 1993. Water condensation promotes fungal growth in ventilation ducts. Indoor Air 3: 106–112.

8 Price, D.L., D.L. Ramey, R.B. Simmons, L. Ajello, S.A. Crow and D.G. Ahearn. 1992. Microbial aspects of indoor air quality. In: Proceedings of First Annual IAQ Conference and Exposition, pp. 203–207, National Coalition of Indoor Air Quality, Washington, D.C.

9 Price, D.L., A.D. Sawant and D.G. Ahearn. 1991. Activity of an insoluble antimicrobial quarternary amine complex in plastics. J. Ind. Microbiol. 8: 83–89.

10 Price, D.L., R.B. Simmons, D.L. Ramey, S.A. Crow and D.G. Ahearn. 1993. Assessment of filters treated with a broad spectrum biostatic agent. In: Indoor Air '93 Proceedings. International Conference on Indoor Air Quality and Climate (Seppänen, O., R. Ilmarinen, J. Jaakkola, E. Kukkonen, J. Säteri and H. Vuorelma, eds), Vol. 6, pp. 527–532, Helsinki.

11 West, M.K. and E.C. Hansen. 1989. Determination of material hygroscopic properties that affect indoor air quality. In: IAQ '89 The Human Equation: Health and Comfort, pp. 60–63, American Society of Heating Refrigeration and Air Conditioning Engineers Inc., Atlanta, GA.