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Fungal colonization of air filters for use in heating, ventilating, and air conditioning (HVAC) systems

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SUMMARY

New and used cellulosic air filters for HVAC systems including those treated with antimicrobials were suspended in vessels with a range of relative humidities (55–99%) and containing non-sterile potting soil which stimulates fungal growth. Most filters yielded fungi prior to suspension in the chambers but only two of 14 nontreated filters demonstrated fungal colonization following use in HVAC systems. Filters treated with antimicrobials, particularly a phosphated amine complex, demonstrated markedly less fungal colonization than nontreated filters. In comparison with nontreated cellulosic filters, fungal colonization of antimicrobial-treated cellulosic filters was selective and delayed.

INTRODUCTION

Various components of heating, ventilation and air-conditioning systems such as porous insulation materials, condensation pans, and some painted metal surfaces have been shown to serve as foci for the amplification of fungi [1,10,11,13]. Certain of the fungi from buildings with a history of complaints of unsuitable air quality can produce a variety of volatile organic compounds [3,5]. In most cases, fungi enter buildings with outside air. Mechanical filtration media employed in commercial buildings for removal of particulate matter from incoming air are commonly panel filters composed of cellulose fibers (about 70%) mixed with various polyester fibers in a cardboard frame. The fibers are bound by mastics such as polyvinyl alcohol or ethylene vinyl chloride and the surfaces are pleated to increase filtering area. These type panel filters have a poor removal efficiency for particles smaller than 5 μm in diameter. Both live and dead fungal propagules in indoor air may pass through such filters and may be associated with hypersensitivities and sometimes, in the case of viable fungi, infections [9].

Filters when dampened may support fungal growth which could result in the destruction of the filter and/or growth of the mycelium through the filter with eventual release of conidia on the downstream side [7]. Endemic aspergillosis caused by *Aspergillus fumigatus* and *Aspergillus flavus* has been associated in a hospital with heavy growth of the fungi in air conditioners or on the air filters [2,8]. Particle board frames of the filters were most heavily involved, but fungal growth was observed also on the pleated filter medium [2]. Droplets of condensate blown from the cooling coils were thought to be the source of moisture that dampened the filters.

Price et al. [12] compared various polyester and microglass air filter media, untreated and treated with a phosphated quaternary amine complex, for their ability to support the growth of fungi. Untreated air filter media were susceptible to colonization by fungi within 30 days. Recently, cellulosic filter media treated with this antimicrobial and a similar filter medium treated with a silane-quaternary amine inhibitor have become available [6]. We have examined cellulosic air filter media treated with the above antimicrobials for their ability to inhibit fungal growth.

MATERIALS AND METHODS

Two types of cellulosic filter media, one without and one treated with a phosphated quaternary amine complex (PQ) were obtained directly from the manufacturer (AAF International, Louisville, KY, USA). A second cellulosic medium treated with a silane-quaternary amine (SQ) was obtained from retail sources. These three types of filters were examined for their susceptibility to fungal colonization as follows.

Initial culture

Filters were cut into sections (1–4 cm^2) and were used as a source of inoculum for various enriched agars. Agars used included Mycological Agar (Difco, Detroit, MI, USA) with 0.5 g L^{-1} chloramphenicol, as well as Czapek's Agar with sucrose or glycerol. The agars, at about 46 °C, were poured through the sections of filters that lay in standard plastic petri dishes. The agars were poured to a depth that just covered the surface of the filter section. The plates were incubated for up to 10 days at 20–24 °C.

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Filter banks

The cellulosic filter panels (61 cm × 61 cm × 5 cm) were placed onto the primary filter bank (36 filters) of a new institutional building in metropolitan Atlanta, GA, during October 1993, and maintained for at least 40 days. At 7-day intervals up to 42 days the filters were removed from the primary filter bank and cut into sections (4 cm^2). Several sections were examined microscopically for fungi; others were cultured as described above.

Chamber studies

Sections (4 cm^2) of new and used filters were suspended in glass chambers (4 L) containing non-sterile potting soil, the presence of which stimulates fungal growth, a sterile beechwood control strip, and different saturated salt solutions to give a range of relative humidities from about 55% to 90% [4]. Unused and used sections were held in control chambers without soil reservoirs at >90% relative humidity for over 90 days. The chambers were kept at 22–24 °C. At 7-day intervals, sections from the chambers were examined microscopically for evidence of fungal colonization (observation of extensive hyphae and/or mature conidiophores and conidia).

Identifications of fungi on the filters were based on comparisons of reproductive structures observed directly on the filters with those developed by isolates picked from the filters and placed in pure culture. Identifications were based on standard morphological and physiological procedures. Sections of filter media were examined visually with a Hirox video system (Hi-Scope Systems Co., Haworth, NJ, USA). Acetate tape mounts were prepared from filter media sections and examined for microscopic colonization using an Olympus (Olympus Corp., Lake Success, NY, USA) BH-2 light microscope fitted with Nomarski DIC optics. Specimens for scanning electron microscopy (SEM) were mounted directly onto SEM stubs, sputter coated with Au/Pd with a Denton (Moorestown, NJ, USA) Desk II sputter coater, and examined in a Leica (Cambridge, UK) 420 scanning electron microscope.

RESULTS

Initial culture

All sections from untreated filters yielded fungi on initial culture, mostly less than five colonies per section, but ranging up to 20 colonies. *Aspergillus niger* was the predominant species isolated, but several colonies of *A. fumigatus* and *Rhizopus* sp. were obtained. Initial cultures of unused PQ filter medium yielded only a few colonies, typically one to three per plate of *Paecilomyces variotii, Pithomyces chartarum*, and *Penicillium* sp. Sections of the unused SQ filter medium yielded low numbers of colonies (<12) per plate. The diversity of species from the SQ filter medium was greater than that obtained from the PQ medium. *Aspergillus niger* was most common, but *A. versicolor, A. fumigatus, Dreshlera* sp., *Paecilomyces variotii, Penicillium corylophylum, P. sclerotorum, Pestalotia* sp., and *Trichoderma viride* were isolated. Although isolation of fungi from the unused filter media was common, there was no microscopic evidence of active colonization. Fungi isolated from unused filter media of each type are listed in Table 1.

TABLE 1

Initial culture of unused filter media

	Untreated	PQ-treated	SQ-treated
Fungi isolated	*Aspergillus niger* *A. fumigatus* *Rhizopus* sp. *Paecilomyces variotii* *A. flavus* *A. versicolor* *Trichoderma viride* (above 90%) *Cladosporium* spp. *Alternaria alternata* *Curvularia lunata*	*Paecilomyces variotii* *Pithomyces chartararum* *Penicillium chrysogenum*	*A. niger* *A. fumigatus* *A. versicolor* *Dreshlera* sp. *Paecilomyces variotii* *Penicillium corylophylum* *P. sclerotorum* *Pestalotia* sp.

Filter panels $n = 5$.

Filter banks

Untreated filter medium from the filter banks, on direct microscopic examination, demonstrated numerous conidia of a variety of fungi, but only two sections showed microscopic colonization, apparently by *A. niger* and *A. flavus*. These untreated sections from the filter banks, when placed in the chambers with soil, all developed heavy colonization by a variety of species including: *A. niger, A. flavus, A. versicolor, A. ustus, Penicillium* sp. (subgenus *Penicillium*), *P. chrysogenum, Cladosporium herbarum* and *Alternaria alternata* (Fig. 1).

Neither PQ- nor SQ-filter media from the filter banks gave evidence of colonization. Upon secondary exposure of SQ filter medium in the chambers, however, dense growth of *Aspergillus* spp. developed particularly along the outlines of where the medium had been glued to the metal support grid (Fig. 2). PQ medium from the filter banks, beginning at about day 14 and after secondary exposures up to 28 days in the chambers, showed extensive fungal colonization by *Acremonium* sp. Other sections of the PQ filter medium remained uncolonized. Overall incidences of the fungi among the test systems are summarized in Table 2.

Chamber studies

In the chambers, all untreated sections at relative humidities above 70% showed microscopic colonization by 14 days, mostly by *A. niger, A. versicolor,* and *Cladosporium* spp. Cultures of all the untreated sections yielded confluent growth of fungi. Only one of the 14 sections of the PQ filter medium was colonized after 14 days in the chambers. By day 28, a second PQ filter section developed a sector colonized again by *Cladosporium herbarum*. Non-colonized sections of PQ filter medium in the same chamber remained free from colonization. Most of the SQ filter medium samples showed scattered darkened patches of colonization by *A. niger* and *P. variotii*, as well as two occurrences of *A. fumigatus*. By 28 days conidi-

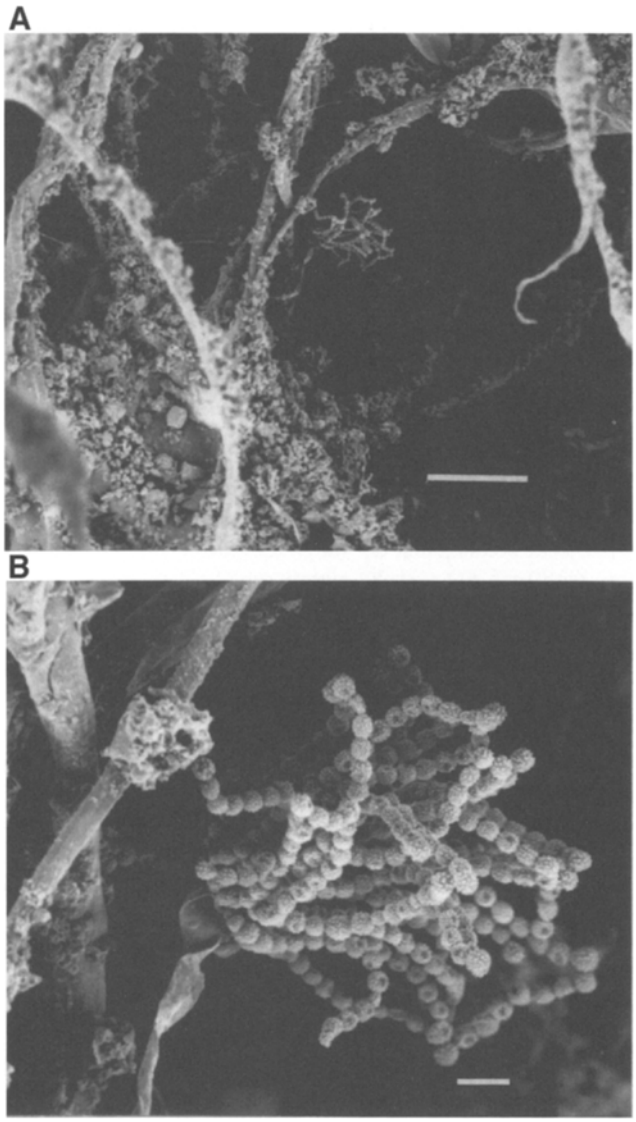

Fig. 1. (A & B) Hyphal elements and mature conidiophore with conidia on untreated cellulosic filter media after 90 days of use followed by 14 days challenge in a laboratory chamber. (A) Bar = 100 μm. (B) Bar = 10 μm.

ophores and conidia of *A. niger* were observed on all SQ samples (Fig. 3).

Controls

Fungal growth was observable with the unaided eye on the beechwood control strips within eight days in chambers with

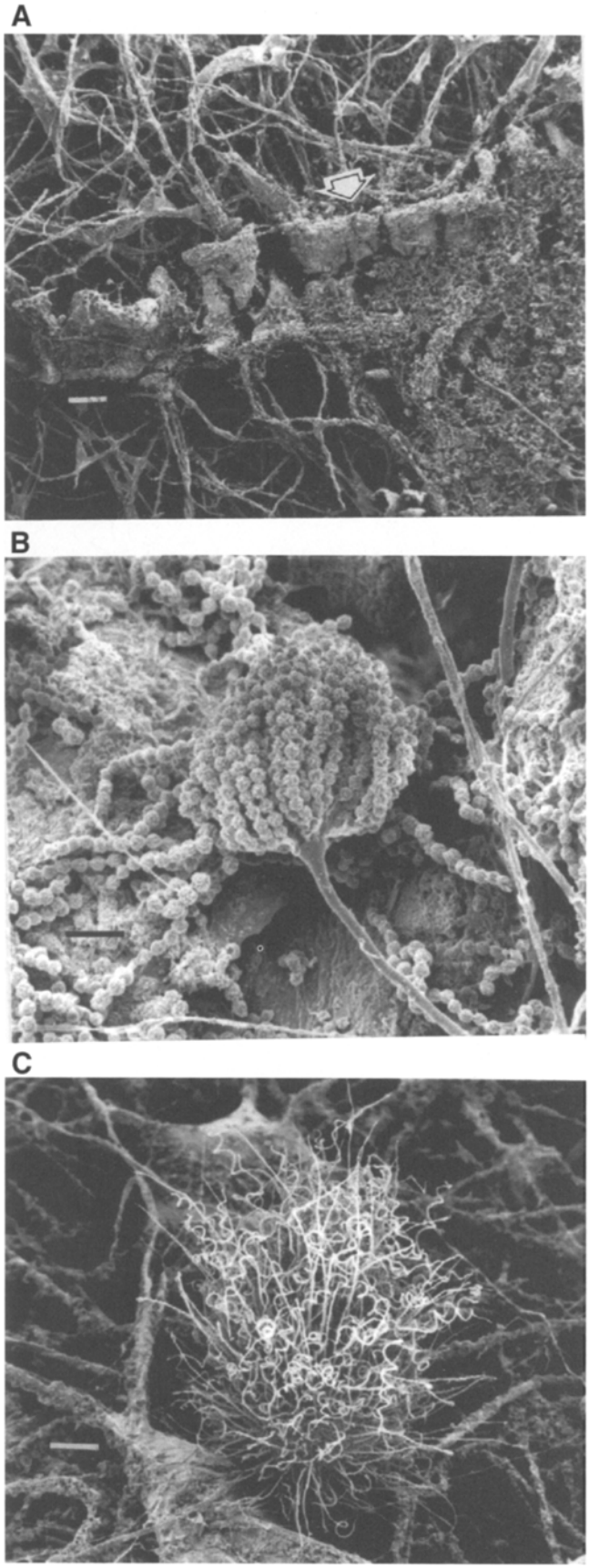

Fig. 2. (A) SQ-treated cellulosic filter media after 40 days use and 14 days challenge in a laboratory chamber. The primary colonization sites for the fungi appear to be associated with the glue used to attach the media to the wire support scrim. Bar = 200 μm. (B) Mature conidiophore of *Aspergillus* sp. observed in association with the glue. Bar = 10 μm. (C) *Chaetomium* sp. found on used SQ-treated filter media after 40 days use and 28 days challenge in a laboratory chamber. Bar = 100 μm.

TABLE 2

Fungal incidence and colonization of cellulosic air filter media[a]

	Untreated	PQ-treated	SQ-treated
Initial culture unused filter media	6/6[b]	6/6	17/18
Microscopic colonization in challenge chambers[c]	12/12	1/14	34/51
Colonization in filter banks by 30 days	2/14	0/14	0/14
Colonization in chamber —post filter banks	18/18	9/18	6/6

[a] Filter panels $n = 5$.
[b] No. filter sections positive/no. tested. Filter Panels $n = 5$.
[c] After 14 days.

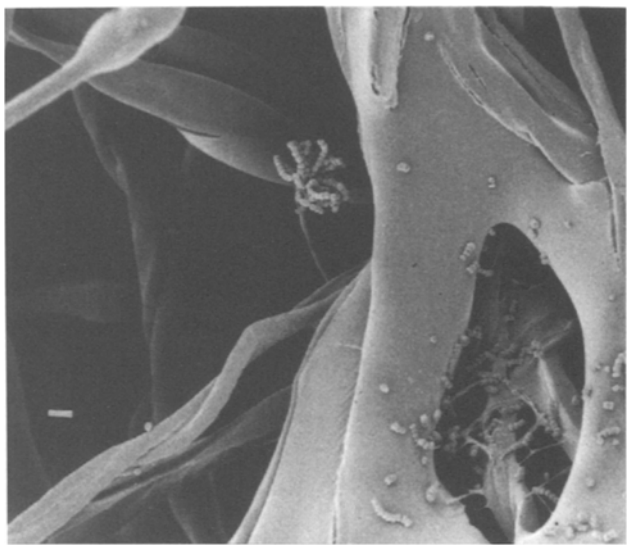

Fig. 3 *Aspergillus niger* conidiophore and conidia on unused SQ-treated media after 18 days challenge in a laboratory chamber. Bar = 10 μm.

soil at all relative humidities above 70%. Between 14 and 28 days sporadic areas of dark discoloration were noted also on the unused non-treated cellulosic filter medium and more rarely on the PQ- and SQ-filter media. Beechwood and filter media in chambers without soil reservoirs did not show visually detectable growth after a 90-day test period, but microscopic colonization, mostly by *Aspergillus niger*, was observed on untreated filter sections by 28 days. Untreated sections of used filter material placed into control chambers without soil also developed heavy colonization, but over a longer time period than observed in the presence of soil.

DISCUSSION

Cellulosic fiber media are the most commonly used type of primary HVAC filter found in the USA. Though the colonization of cellulosic filters by fungi while in use in HVAC systems may not be common in a well-designed and well-maintained system, it has been reported to occur [2,7]. All of the unused filter media examined in this study yielded fungi upon culture. These fungi may have represented the fortuitous occurrence of conidia but in some instances fungal conidia were observed within the matrix on initial observation of the filters. Perhaps these conidia resulted from brief periods of fungal growth in the filter during transportation and storage. Our two instances of colonization of filters in filter banks followed periods of heavy rainfall and temperatures of 22–26 °C. Arnow et al. [2] related an episode of endemic aspergillosis in a hospital to the growth of *A. fumigatus* on HVAC air filters. The filter frames and filters were thought to have been dampened by droplets of condensate blown from the cooling coils located immediately upstream.

Testing of filter media in chambers with varying moisture levels has shown that the basic cellulosic filter medium is susceptible to colonization by fungi. Cellulosic filter media treated with both PQ and SQ antimicrobial treatments, as compared to untreated medium, showed delayed colonization and reduced densities and varieties of recoverable species. In general, PQ-treated filter medium was more recalcitrant to fungal colonization than was SQ-treated filter medium.

Untreated filter media in an ideally operated HVAC system will probably not be colonized by fungi within a 'normal' three month use-life. Our studies indicate that filters exposed to volatiles from soil and moisture may have a considerably shorter life. Standing water near to rooftop HVAC intakes and soil and moisture in basements or crawlspaces may contribute to loss of recalcitrance to microbial contamination of air filter media.

Variation in the colonization of individual samples of the treated filter media suggests that the antimicrobial compounds were not applied uniformly to the surface of the filter media. Areas free from inhibitor, including the frames, could lead to a localized breakdown of the efficacy of the filter unit.

Within the scope of this project, both antimicrobial compounds tested prevented colonization of the filter media during their use and delayed colonization in subsequent laboratory tests. Antimicrobial treatments of air filters may reduce the potential of fungal colonization and subsequent adverse health effects.

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REFERENCES

1 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: 277–280.
2 Arnow, P.M., M. Sadigh, C. Costas, D. Weil and R. Chudy. 1991.

Endemic and epidemic aspergillosis associated with in-hospital replication of *Aspergillus* organisms. J. Infect. Dis. 164: 998–1002.

3 Bayer, C.W. and S.A. Crow. 1993. Odorous volatile emissions from microbiological contamination. In: IAQ 93 Environments for People, pp. 99–104. American Society for Heating, Refrigeration, and Air Conditioning Engineers, Atlanta, GA.

4 Ezeonu, I.M., J.A. Noble, R.B. Simmons, D.L. Price, S.A. Crow and D.G. Ahearn. 1994. Effect of relative humidity on fungal colonization of fiberglass insulations. Appl. Environ. Microbiol. 60: 2149–2151.

5 Ezeonu, I.M., D.L. Price, R.B. Simmons, S.A. Crow and D.G. Ahearn. 1994. Fungal production of volatiles during growth on fiberglass. Appl. Environ. Microbiol. 60: 4172–4173.

6 Gettings, R.L., R.A. Kemper and W.C. White. 1990. Use of an immobilized antimicrobial for intervention of environmental sources of microbial populations in the homes of mold-sensitive subjects and subsequent monitoring of the presentation of allergic symptoms. Dev. Ind. Microbiol. (J. Ind. Microbiol., Suppl. 5, G.E. Pierce, ed.) 31: 231–244.

7 Kuehn, T.H., D.Y.H. Pui, D. Vesley, C.D. Berg and M. Peloquin. 1991. Matching filtration to health requirements. ASHRAE Trans 97 Pt 2. American Society for Heating, Refrigeration, and Air Conditioning Engineers, Atlanta, GA.

8 Lentino, J.R., M.A. Rozenkranz, J.A. Michaels, V.P. Kurup, H.D. Rose and M.W. Rytel. 1986. A retrospective review of airborne disease secondary to road construction and contaminated air conditioners. Am. J. Epidemiol. 116: 430–437.

9 Mishra, S.K., L. Ajello, D.G. Ahearn, H.A. Burge, V.P. Kurup, D.L. Pierson, D.L. Price, R.A. Samson, R.S. Sandhu, B. Shelton, R.B. Simmons and K.F. Switzer. 1992. Environmental mycology and its importance to public health. J. Med. Vet. Mycol. 30(suppl. 1): 287–305.

10 Morey, P.R., M.J. Hodgson, W.G. Sorenson, G.K. Kullman, W.W. Rhodes and G.S. Visvesvara. 1986. Environmental studies of moldy office buildings. ASRHAE Trans 92: 399–419. American Society for Heating, Refrigeration, and Air Conditioning Engineers, Atlanta, GA.

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

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

13 Price, D.L., R.B. Simmons, I.M. Ezeonu, S.A. Crow and D.G. Ahearn. 1994. Colonization of fiberglass insulation used in heating, ventilation, and air conditioning systems. J. Ind. Microbiol. 13: 154–158.