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SPECIAL TOPIC: DISINFECTANTS AND MICROBIAL CONTROL

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Mold colonization during use of preservative-treated and untreated air filters, including HEPA filters from hospitals and commercial locations over an 8-year period (1996–2003)

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Abstract High efficiency particulate arrestance (HEPA; 99.97% efficient at 0.3 μm) filters, filters with ASHRAE particulate arrestance rating of 90–95% at 1 μm (90–95% filters), and lower efficiency cellulosic-polyester filters from air conditioning systems in hospitals and commercial buildings were removed from the systems and examined microscopically for mold colonization. Cellulosic-type filters from systems with water entrainment problems typically were colonized, or became colonized upon incubation in moisture chambers. Species of *Acremonium*, *Aspergillus*, and *Cladosporium* were most common. With air filters of all types, treatment of filter media with an antimicrobial preservative tended to reduce or delay colonization. Mold colonization of HEPA and 90–95% filters was observed most often on the load surfaces, but two untreated HEPA filters were permeated with fungi, one with *Aspergillus flavus*, the other with *Cladosporium* sp. Air filters in heating, ventilating, and air conditioning (HVAC) systems, particularly those with chronic or periodic exposure to moisture, may serve as point sources for indoor molds.

Keywords HEPA air filters · Fungal colonization · Hospitals · Phosphated amine preservative

Introduction

Air filters used in heating, ventilating, and air conditioning (HVAC) systems most probably contain fungal propagules prior to their installation and, once in use, the filters are exposed to an onslaught of fungi from the environment [7]. In moist environments the cellulosic filter medium and cardboard frames of commonly used air filters serve as excellent substrata for growth of numerous molds, whose conidia may be released into the downstream air [3, 7, 8]. Numerous molds may be isolated in high densities from filter media and frames, but these may be species different from those that are actually colonizing the filter surfaces, or they may represent only a portion of the colonizing species [2]. We define colonization on the basis of microscopic observation of mycelial development with conidiogenesis on the substratum. Unacceptable levels of mold amplification on moist cellulosic-type air filters and eventual downstream release of conidia may result in malodors and/or induce occupant hypersensitivities. In extreme cases of chronic moisture exposure, this may occur even when preservatives designed to delay or inhibit such events are employed [7], but the potential for fungal amplification and penetration of high efficiency particulate arrestance (HEPA) filters whose components are recalcitrant for fungal growth is generally not recognized. This report updates our ongoing comparative studies on mold colonization of preservative-treated and untreated air filters in air distribution systems in hospitals and commercial buildings, with inclusion of observations on colonization of HEPA filters.

Materials and methods

A total of 188 air filters, including 45 from 29 different hospitals and from various commercial buildings, mostly from the southeastern United States, were removed from filter banks, placed in clean plastic bags and shipped to our laboratories. The filters were mostly whole, but portions of filters were also received, particularly when multiple samples were requested from a single filter bank. At initial collection, filters were chosen mainly on the basis of visual evidence of soiling or as representative

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of filters in a bank at a scheduled replacement time. Multiple sites on the filters were examined microscopically, usually within 72 h of collection, via clear-polypropylene-adhesive tape mounts and scanning electron microscopy. Sections of the filters were cultured onto enrichment culture media for isolation and identification of predominant fungi. Sections were also placed in humidity chambers for subsequent periodic examination for colonization. At three sites (selected on the basis of preliminary data detailed in the text), we installed additional filters in the banks and periodically examined the filters for developing fungi. Details of all procedures and types of filters are described elsewhere [4, 6–8]. The time-course of the observations encompasses the years 1996–2003.

The preservative used for the majority of treated filters was Intersept, a non-toxic, phosphated amine complex of low water solubility with EPA registration for use in air conditioning systems and carpets [5].

Results

Microscopic observations of mold colonization of used air filters removed from office buildings and hospitals over an 8-year period suggested that treatment of air filters with a preservative could reduce or delay the onset of mold growth on the filters (Table 1). This potential value of a preservative treatment was further supported by the fact that nearly all samples from untreated cellulosic air filters, which initially appeared negative for mold colonization, developed readily detectable colonization following a 2- to 3-week incubation at high humidity. Six filters with ethylene vinyl chloride binders remained negative for colonization over this period. Filters that had been preserved with Intersept typically resisted detectable colonization for periods exceeding 3–4 weeks in humidity chambers.

At one hospital located on the coast of the Gulf of Mexico, both primary and secondary cellulosic filters were frequently moist (occasionally dripping wet) during warm-humid weather. The beverage board frames were overgrown with bacteria and fungi, particularly species of *Acremonium*, *Aspergillus*, and *Penicillium*, within several weeks of their installation. Filter efficiency was rapidly reduced because of sagging of the beverage board frames due to loss of structural integrity.

At a second hospital, an in situ test series was conducted in a secondary filter bank downstream of the condensation coils. Preserved air filters whose beverage board frames had been coated with a diluted stryrenated-acrylic containing 3% of the phosphated amine were compared with control filter frames with no coating. The phosphated amine-treated acrylic coating had been brush applied to the beverage board filter frames and allowed to air dry. The filters were monitored on a bi-weekly basis for visual evidence of fungal colonization. Visually detectable fungal growth was first observed on untreated filters within 4–6 weeks in the moisture-laden HVAC system with operating temperatures of 17–20°C. At 12 weeks, all the filters were bagged and returned to the laboratory for analysis. Untreated sections of the beverage board filter frame were colonized with dense populations of *Alternaria*, *Cladosporium*, and *Chaetomium*. Exterior sections of the filter frame coated with the preserved clear acrylic coating were free of fungal colonization; however, interior sections where the air filter medium was glued to the frame were colonized with the above-cited fungi as well as with sporadic occurrences of colonies of *Aspergillus* and *Penicillium*.

Multiple filters were also installed and examined periodically in a third hospital, which had an episode of endemic aspergillosis among transplant patients. At all observations, the filters appeared relatively clean and dry and there was no evidence of fungal colonization of filters or other components of the air conditioning system. Point sources of a variety of fungi (*Aspergillus versicolor*, *Acremonium*, *Chaetomium*, *Cladosporium*, and Stachybotrys) were found within the hospital around leaking windows or on wallboard near plumbing leaks, and heavy densities of *Aspergillus fumigatus* (the aetiological agent identified as responsible for the endemic infection by the clinical laboratory) were isolated from wood chip flower mulch outside entrance doors to the hospital. In enrichment culture on malt extract agar, the wood chip flower mulch grew a virtual monoculture of *A. fumigatus*.

HEPA and 90–95% filters with galvanized steel or wood laminate frames and microglass filtration media are designed for extended use in environments that require a high degree of hygiene and airborne particulate control. These filters typically resist colonization because of the refractive nature of their components. Nevertheless, 5 of 24 of the 90–95% filters and 3 of 21 HEPA filters were colonized with fungi upon initial microscopic examinations (Table 1). Sealants, mostly on load surfaces, showed microcolonization, but two untreated HEPA filters from different hospitals were colonized both on load and supply surfaces, one by *Cladosporium* sp. (Fig. 1) and the other by *Aspergillus flavus*, a species not uncommon on cellulosic filters from several sites [2].

Table 1 Incidence of mold colonization observed on initial examination of selected air filters from hospitals and office buildings[a]

	Untreated filters	Preservative treated filters
90–95%	25% (1/4)[b]	20% (4/20)
HEPA (99.97E)	33% (2/6)	7% (1/15)
Total filters[c]	26% (28/109)	16% (13/79)

[a]Colonization based on microscopic observation of fungal mycelium and conidiogenesis upon receipt at the laboratory. Air filters selected from filter banks between 1996 and 2003 on visual evidence of soiling or as visually representative of filters at scheduled replacement times. The collection, handling of samples, the exact type of filter and its duration of use varied

[b]Number colonized/number examined

[c]Includes cellulosic and polyester filters

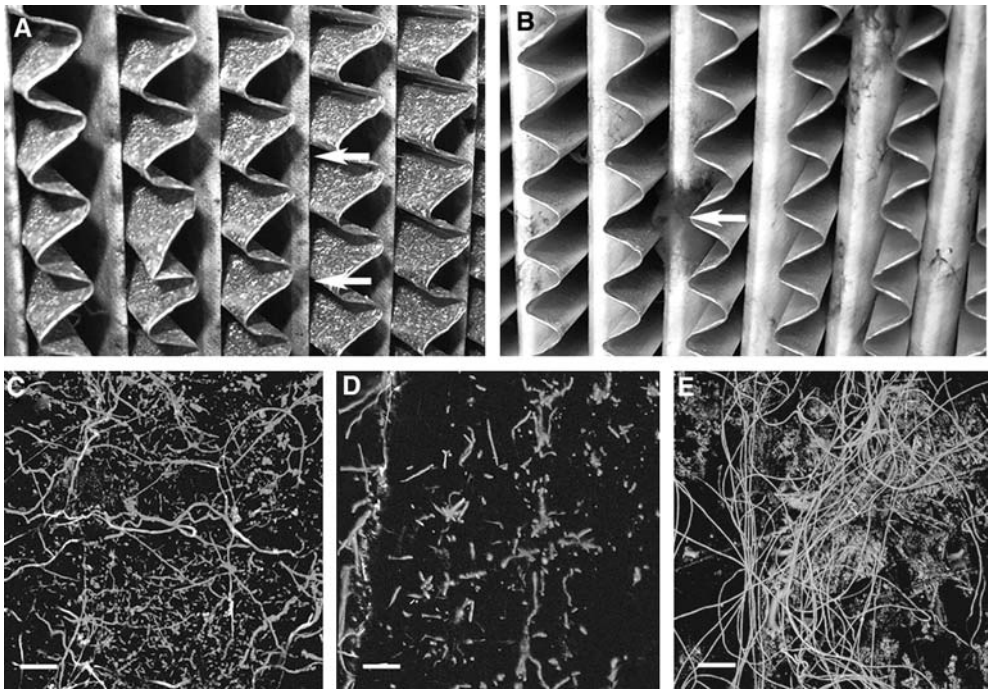

Fig. 1a–e Fungal growth with penetration of microglass filter medium of a galvanized steep-framed HEPA filter. **a** Load side of filter showing discolored patches on the filter material (*arrows*). **b** Supply side of filter showing brown fungal colony on the surface (*arrow*). **c** Load side of filter stained for fungi (laser confocal microscopy) showing hyphal elements on the filter medium surface. **d** Cross section of filter medium stained for fungi (laser confocal microscopy) showing hyphae penetrating the filter matrix from the load side (*left*) towards the supply side. **e** Supply side of filter stained for fungi (laser confocal microscopy) showing hyphal elements on the filter medium surface

Discussion

The dense fungal colonization on HEPA and 90–95% filters is of particular note because the metal-microglass components cannot support fungal growth. Although it is recognized that fungi may indeed grow on adsorbed organics on the load side of a HEPA filter, penetration and proliferation on the supply side has not previously been noted. Two of the HEPA filters we examined, one colonized with *Cladosporium* sp, the other with *A. flavus*, demonstrated fungal growth throughout the filter. We have previously noted that metal surfaces and fiber-glass insulation in air conditioning systems serve as substrata for fungi growing on adsorbed nutrients [1].

The filters examined 1994–2003 represent a non-controlled selection with a bias for facilities with mold or moisture problems, which negates determination of the actual frequency of fungal colonization of filters in hospitals or commercial buildings. Moreover, the incomplete preservative treatment of the frames of cellulosic-type filters limited our assessment of the benefit of the preservative treatment to delay the onset of fungal colonization. A 2-fold benefit is suggested for the preservative-treated acrylic used on the filter frames: moisture exclusion and chemical inhibition of microbial growth. The phosphated amine antimicrobial present in most of the treated filters we examined expresses mainly biostatic properties, although contact may result in germinating conidia of some species undergoing lysis [5].

The in vitro and in situ observations of delayed colonization of preservative-treated versus untreated filter media, particularly by medically important aspergilli, warrant continued evaluative studies of a prospective nature. Our studies further indicate that air filters with chronic or periodic exposures to moisture may, at least, serve sporadically as point sources of indoor molds.

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