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Evaluation of Fungal Growth on Fiberglass Duct Materials for Various Moisture, Soil, Use, and Temperature Conditions

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Abstract Fiberglass duct materials are commonly used in both residential and commercial heating, ventilation, and air-conditioning (HVAC) systems to provide the needed thermal insulation and noise control. Many building investigations have documented biocontamination of these materials, and the appropriateness of their use in high humidity locations has come into question. A series of experiments, each lasting 6 weeks, was conducted in static environmental chambers to assess some of the conditions that may impact the ability of a variety of fiberglass materials to support the growth of a fungus, *Penicillium chrysogenum*.

Three different fiberglass duct liners (FDL), one fiberglass duct board, and fiberglass insulation, all newly purchased, were obtained as were samples of used (>5 years old) materials. Samples of these materials were tested to evaluate the effects of moisture, soil, use, and temperature on their ability to support the growth of *P. chrysogenum*. These studies demonstrated that *P. chrysogenum* could amplify under conditions of low (12°C) and room (23°C) temperature and high relative humidity on samples of one of the newly purchased materials, and that either wetting and/or soiling increased the materials' susceptibility. *P. chrysogenum* was able to grow on all the used material samples. While the results of this study apply directly only to fiberglass duct materials, they suggest that dust accumulation and/or high humidity should be properly controlled in any HVAC duct to prevent the growth of *P. chrysogenum*.

Key words Biocontaminant; Mold; Materials; Fiberglass; Indoor air quality; Dust.

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Introduction

Estimates of biocontamination in problem buildings range from 5% (Wallingford and Carpenter, 1986) to 45% (Woods, 1989). Source management is critical to the prevention and control of biocontamination. Case studies of problem buildings have shown that the locations for source reservoirs of biocontaminants, especially fungi, are usually the building itself, specifically the materials from which the building is constructed or with which the building is furnished (Morey, 1993; Hunter et al., 1988). The types of materials that may become sources include: structural components, interior construction and finishing materials, thermal and moisture protection materials, furnishings, and mechanical systems. Once a source reservoir is established, dissemination to other parts of the building may be imminent, particularly if the source is associated with the heating, ventilation, and air-conditioning (HVAC) system.

Understanding the conditions that allow establishment of sources is important. Many factors contribute to the potential of a material to become a source. It is well established that microorganisms will grow, given sufficient nutrients, water, and temperature. What is less clear is exactly what this means in a HVAC system. Moisture may be available through standing water, water incursion, condensation, or adsorption. Nutrients can be provided by the material itself or by accumulated dust deposited on the surface of the material. It has been hypothesized that accumulated soil increases a material's hygroscopicity, while providing a nutrient source (West and Hansen, 1989). While eliminating all biocontaminants in a building will never be possible, understanding the conditions that promote fungal growth may limit the development of source reservoirs.

Thermal insulation and noise control are required for the HVAC system to be energy-efficient and commercially acceptable. Fiberglass materials have been used as an effective solution for both problems. However, HVAC sites and materials that have been identified as problems and emission sources in HVAC systems in-

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clude a number of fiberglass materials (Abdou and Sando, 1994; Batterman and Burge, 1995).

Fiberglass duct materials have been reported as sources of biocontaminant emissions, and it has been suggested by Morey and Williams (1991) that "the continued use of porous insulation appears to be incompatible, from a microbiological point of view, with the concept of a healthy building". In a study of 18 buildings with documented microbial contamination, manufactured porous insulation was shown to be a source reservoir in 9 of those buildings (Morey, 1988). In one particular building in that study, the debris on the surface of the air-handling unit contained approximately 1 million viable fungi/g, of which 98% were *Penicillium*. The air at this site contained over 3000 colony-forming units (CFU)/m^3 of *Penicillium*, more than 10 times the level outdoors. In another study of a residence where occupants complained of an intermittent odor, colonization of duct liner was found in a relatively well maintained HVAC system (Ahearn et al., 1993). Ezeonu et al. (1994) isolated heavily mold-contaminated fiberglass duct liners and duct boards from eight buildings where the occupants complained of moldy odors. In some cases, the reasons for the problems were obvious (often poor design, maintenance, or filtration), but in others no cause was apparent. Consequently, considerable controversy exists concerning the proper use of this material and other HVAC duct materials.

A shortage of comparable data is one of the biggest problems facing researchers developing prevention and control strategies for indoor biocontaminant pollutants. Most data are from case studies of operating buildings. Building conditions (temperature and humidity) vary so extensively that conclusions based on case studies are comparisons of dissimilar measurements. The industry has no economical substitute material, and engineering alternatives may be difficult to implement and have their own indoor air quality (IAQ) effects. Systematic reproducible studies of biocontaminant growth on materials may provide data that can improve materials and help identify engineering alternatives. Previous studies using ceiling tile materials have shown the importance of both the composition and the hygroscopicity of the materials in their ability to support fungal growth (Foarde et al., 1993).

The objectives of this study were to quantitatively evaluate, under controlled reproducible conditions, the effects on fungal growth of a variety of environmental conditions commonly thought to influence fungal growth on fiberglass duct materials. This series of experiments, employing static chambers with known environmental conditions, was designed to evaluate the impacts of moisture, soil, use, and temperature. Through these experiments we hoped to begin to answer the following questions:

1) Under what conditions are fiberglass duct materials able to support microbial growth? and
2) Will high relative humidity (RH) alone support microbial growth, or is wetting and/or soiling also required?

A detailed description of the test method and the results of the four groups of experiments are given below. The first group of experiments examined the impact of moisture on newly purchased fiberglass duct materials. Two moisture-related situations were investigated: 1) microbial growth on materials exposed to high RH (97%) alone or following wetting of the materials with liquid water, and 2) comparisons of growth on materials equilibrated to four different RH levels. The second group of experiments investigated the impact of soiling new duct materials on microbial growth at high equilibrium RH levels. Growth at two levels of soiling – moderate and heavy – was evaluated. The third and fourth experiments examined growth on used fiberglass duct materials and the effects of temperature on growth on a single material, respectively.

Methods and Materials

The basic experimental method consisted of quantitatively evaluating the growth of a single fungus on small samples of conditioned test materials under static equilibrium RH conditions. The various aspects of the experiment are described below.

Static Chamber

Static chambers (32×39×51 cm), prepared by modifying acrylic-walled desiccators, were used to provide controlled environments for the fungal growth tests (Foarde et al., 1992; Foarde et al., 1994). The chambers were placed in a dark, temperature-controlled (21±3°C), HEPA (High Efficiency Particulate Absolute) -filtered room. Each static chamber was equipped with three shelves, a bottom tray containing a saturated salt solution, and a hygrometer.

Saturated salt solutions were used to maintain specific RHs (ASTM 104-85) within each chamber. The RH values and saturated salt solutions used to attain them were: 54% RH - magnesium nitrate, 85% RH - potassium chloride, 90% RH - barium chloride, 94% RH - potassium nitrate, and 97% RH - potassium sulfate (Greenspan, 1977).

Duct Materials Tested

The newly purchased duct materials tested were samples of three brands of fiberglass duct liner, one brand

Table 1 Composition of test materials

Material	Composition
Fiberglass duct liners A and C (FDL-A and FDL-C)	>44–98% fibrous glass, 1–18% urea polymer of phenol and formaldehyde or urea-extended phenol melamine-formaldehyde resin, <0.1% formaldehyde
Fiberglass duct liner B (FDL-B)	82–98% fibrous glass, 2–18% urea-extended phenol-formaldehyde resin (cured) or urea-extended phenol-melamine-formaldehyde resin (cured), <1% non-woven, Foil-Skrim-Kraft or vinyl facings or vinyl or latex coatings
Fiberglass duct board	85–96% fibrous glass wool, 4–15% cured binder, <1% formaldehyde
Fiberglass insulation	90–95% refractory ceramic fiber, 0–10% phenol formaldehyde

of fiberglass duct board, and one brand of fiberglass insulation from a flexible duct. All new duct materials were purchased from local commercial vendors. The actual test pieces, 3.8 cm squares, were cut from these samples. The compositions of the new materials compiled from the Material Safety Data Sheets are summarized in Table 1. Although fiberglass duct liners (FDL) manufactured by three different companies were used as test materials, the composition of two (A and C) was similar, and one description encompasses them both. FDL-B contained what was described in the product literature as a "permanent biocide" in the airstream surface coating. From here on the "permanent biocide" shall be referred to simply as the biocide. All were nominally 2.5 cm thick, and were classed as 24.0 kg/m^3 (1.5 lb/ft^3) in density. In appearance, the duct liners were very similar, with an uncoated surface intended to be attached to a rigid duct material and a polymer-coated surface intended to be in contact with the moving air in the duct.

The fiberglass duct board material was classed as a 72.0 kg/m^3 (4.5 lb/ft^3) material, with a reinforced foil outer coating and a dense but uncoated duct interior surface. The flexible duct insulation was removed from between the interior film lining and the exterior foil/polymer cover.

The used materials were collected by local HVAC contractors from residences having their ductwork replaced. The three types of used fiberglass duct materials were: a duct liner, a fiberglass duct board, and insulation from a flexible duct. While the used materials were chosen to correspond to the new materials and were visually similar, their precise origin could not be determined. There were no reports of complaints or problems associated with the buildings from which the used materials were collected, and they were autoclaved before use to kill any existing microorganisms.

Test Microorganism

Penicillium chrysogenum was selected as the test organism for these studies. It has been reported as one of the most frequently isolated molds from the air, dust, and surfaces of indoor environments (Hunter and Lea, 1995). It has been proposed as a causative agent of allergic alveolitis (Fergusson et al., 1984). In addition, this organism has also been isolated from a number of air-conditioning systems in environments where patients were suffering from allergic disease. Skin and challenge testing against *P. chrysogenum* isolated from these systems yielded more positives than any of the other organisms isolated (Schata et al., 1989).

The *P. chrysogenum* selected as the test organism for these studies was isolated from a contaminated building material and cultivated for use in the laboratory. The culture is being maintained in the University of Texas Medical Branch Fungus Culture Collection as UTMB3491.

Moisture Content

Two types of measurements are commonly used to evaluate building material moisture: most engineers think in terms of moisture content (MC) and many microbiologists utilize water activity (a_W). Both measurements were included in this study, as discussed below.

MC, defined as mass of water per unit mass of dry material, is measured gravimetrically (West and Hansen, 1989; Foarde et al., 1993). It is a bulk measurement of the water in a sample of the material. Dry material, in this context, has had adsorbed (bound by molecular bonds) and absorbed (held loosely in capillary spaces) water removed through normal oven drying methods (105°C), but may retain strongly chemically bound water (Richards et al., 1992; Flannigan, 1992). An alternative measure is a_W, primarily used to relate the water content of foods to the ability of microorganisms to grow on them, defined as the equilibrium RH (ERH) above a sample of a material, divided by 100 (Pitt, 1981). In the present context, ERH is the RH in a closed chamber containing a material sample after the material and the air in the chamber have reached water equilibrium. Equivalently, a material may reach equilibrium in a chamber whose RH is held constant by a saturated salt solution. Therefore, the a_W of a material that has been equilibrated in a closed chamber having an RH of 94% is 0.94. Corry (1987) stated that a_W is the proportion of "available water for biological reactions". It is a useful laboratory measurement when RH conditions are known to be at equilibrium.

The term a_W, as used in the literature, is usually reported as the minimum a_W at which an organism can germinate at a given temperature. The minimum a_W for the test organism employed in these experiments is 0.78 (82 days, 25°C) for *Penicillium chrysogenum* (Hocking and Pitt, 1979). Simplistically, this means that *P. chrysogenum* would be expected to grow on nutritionally sufficient materials at water equilibrium with 78% RH or higher. Many factors can affect the minimum a_W for germination of fungal spores, including nutrient availability and temperature (Block, 1953; Pitt, 1981; Flannigan and Miller, 1993).

For porous materials, MC and ERH (or a_W) are related through the material's water adsorption isotherm, and different relationships are obtained for different materials. MC and RH (equilibrium or non-equilibrium) are the measures used in the present study because these terms are more common in the building industry and because, when wetted materials were tested, material moisture conditions were not at equilibrium. For the experiments at equilibrium, a_W is easily calculated.

Artificial Soiling

HVAC dust, collected by a local duct cleaner, was employed to soil the surface of the test materials. All of the dust used in this study came from the same residence. The dust was analyzed using EPA Method 415.2/9060 for total organic carbon, and metals were analyzed by acid digestion subjected to an inductively coupled plasma emission measurement. Table 2 gives the mean composition of the samples. Note that, for this dust, the amounts of total organic carbon, iron, and silicon were approximately equal at between 12 and 16% of the total sample mass.

The dust was applied to the surface of the materials by aerosolizing a known quantity into a dust containment chamber (51×32×39 cm acrylic desiccator) and allowing it to settle onto the materials placed on the bottom of the chamber. The dust was aerosolized using a DeVilbiss Model #175 powder blower. The dust containment chamber was kept in a separate room from the static RH chambers to minimize the potential for cross contamination.

Precut blocks (3.8 cm squares) of the test materials were chamber-conditioned for 3 days at 54% RH, removed from the conditioning chamber, weighed, and placed on a rack in the dust containment chamber. Known amounts of HVAC dust (after passing through a 250 µm sieve) were added to the powder blower and blown into the containment chamber. The dust addition step was repeated until sufficient dust had been added to the system to attain the desired dust loading. The inlet and outlet were plugged and the dust allowed to settle. When the visible dust had settled, the test blocks were removed from the containment chamber, autoclaved, and returned to a sterile 54% RH static chamber. After preconditioning for an additional 3 days at 54% RH, the blocks were removed from the chamber, weighed, and placed in the chamber at the test RH.

Table 2 Composition of HVAC dust used for artificially soiling building materials

Analyte	Mean (mg/g of sample)
Total Organic Carbon	155
Iron	120
Magnesium	4
Zinc	36
Silicon	138

Dust loading was calculated as:

$$M_d = M_2 - M_1 \quad (1)$$

where: M_d = mass of dust on the block, mg
M_2 = mass of block with dust after conditioning 3 days at 54% RH, mg
M_1 = mass of block before dusting after conditioning 3 days at 54% RH, mg.

The mass of dust per 100 cm^2 was determined as:

$$mg\ dust/100\ cm^2 = (M_d/K) \cdot (100) \quad (2)$$

where: M_d = mass of dust on the block, mg
where: K = average surface area of the blocks, cm^2.

Experimental Procedure

For studies under equilibrium or near equilibrium conditions, the sample blocks were chamber-conditioned at the test RH for 3 days before inoculation. Material equilibration studies showed that these particular test materials reached equilibrium MC within 3 days, except for those in the 97% RH chamber in which approximately 90–95% of the final MC was attained in the first 3 days (data not shown). For studies under non-equilibrium conditions, material blocks were wetted with sterile water, and chamber-conditioning was omitted.

To prepare the test organism for inoculation onto the test materials, the organism was first inoculated onto Sabouraud dextrose agar (SDA). The cultures were allowed to grow for 5–10 days until mature confluent growth covered the surface of the plate. A sterile swab wetted with sterile water was gently stroked across the surface of the petri dish to collect the growth. The material collected on the swab was eluted into sterile water. Sterile water was used to minimize the introduction of extraneous nutrients. Microscopic examination of the suspension during the development of the inoculation

protocol demonstrated that the majority of the material consisted of spores. Mycelial fragments were identified in only 2 fields out of 15. The procedure was repeated until a reading of 15%T at 520 nm (Milton-Roy Spec 20D) was achieved (approximately 1×10^7 CFU/ml as verified by dilution and plating). The suspension was mixed well and 10 µl was pipetted on each block of test material for a final inoculum of approximately 1×10^5 CFU per sample. Uninoculated blocks were used as controls. Test blocks (inoculated and uninoculated) were placed in the appropriate static chamber maintained at the specific test RH for incubation.

To quantify the fungal growth, triplicate inoculated and duplicate uninoculated blocks were removed from each chamber for analysis, usually on days 1, 7, 14, 21, 28, 35, and 42. Following removal, the sample blocks were weighed on a Fischer Scientific A200-DS balance to 0.001 g (0.01% of the mean block mass), and placed in sterile receptacles containing phosphate-buffered saline with 0.1% Tween 80. The blocks in buffer were shaken on a wrist-action shaker for 30 minutes, then the block/buffer suspension was diluted and plated on SDA. Plates were incubated at room temperature for at least 1 week. CFUs were counted shortly after visible growth was first noted and again as moderate growth became apparent.

Results and Discussion

As introduced above, the experiments reported below have been organized into four groups to evaluate the effects of moisture, soiling, use, and temperature on the growth of *P. chrysogenum* on the samples of fiberglass duct materials.

Effect of Moisture

Two sets of experiments were performed to evaluate the effect of moisture on the ability of newly purchased duct liners to support the growth of *P. chrysogenum*. In the first set of experiments, samples of all of the test materials were either equilibrated to 97% RH alone or wetted. In the second set of moisture experiments, microbial growth was evaluated on samples of chamber-conditioned FDL-A that were equilibrated in static chambers maintained at 85, 90, 94, or 97%.

High moisture conditions. Figure 1 shows the log CFU change for all five of the test materials between day 1 and day 42 under different treatment conditions. If growth was going to occur within the 6-week time frame for these experiments, it generally had reached its maximum value by day 42. The difference between days 1 and 42 thus is a quantitative measure of the amount of growth expected for these materials. The error bars represent the standard error of the difference

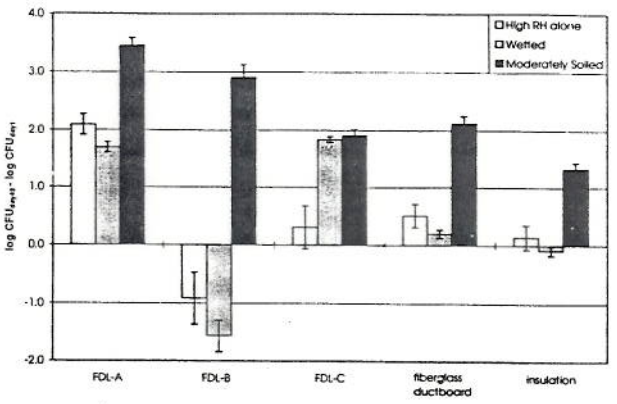

Fig. 1 Growth of *P. chrysogenum* on HVAC materials exposed to high RH alone, wetted, or artificially soiled at 97% RH

between the two means. The first bar (white) for each test material shows the results for samples that were conditioned and maintained at 97% RH. The second bar (gray) for each material gives the results for those samples that were wetted before being placed in the 97% RH chamber. The third bar (black) represents the artificially soiled materials.

As can be seen from Figure 1, of the materials equilibrated in 97% static chambers (white bars), only FDL-A was able to support the growth of the test *Penicillium* within the 6 weeks of testing. FDL-B actually demonstrated a 1 log decrease in CFUs, possibly due to the biocide incorporated into the coating. For this test condition, there was little or no change in the log CFUs for FDL-C, the fiberglass duct board, and the insulation. Note that no change means that the spores with which these samples were inoculated remained culturable but were not exposed to conditions favorable enough to initiate growth.

The effect of wetting the materials (gray bar) also differed depending upon the material. All materials were wetted with 1 ml of sterile water. This resulted in different initial MC, depending on the density of the different test materials. The initial MC (following wetting) was 50% for FDL-A and fiberglass duct board, and 90–100% for the remaining materials. Visually, the samples were very damp, but not dripping wet. Growth on wetted FDL-A increased to the same level of log CFUs as the samples exposed only to 97% RH (without wetting). The decrease in CFUs previously seen on FDL-B maintained at 97% RH was even more notable following wetting. Wetting had the greatest impact on FDL-C. In the previous test with materials maintained at 97% RH, no notable log change was seen in 42 days; however, when wetted, the numbers of *Penicillium* increased by almost 2 logs over the 42 days. The fiberglass duct board and the insulation again showed little or no change in the log CFUs with wetting.

In summary, the impact of moisture differed for the various materials tested. Elevated RH alone was sufficient for growth on FDL-A, while wetting was required for growth on FDL-C. Under elevated RH conditions, FDL-B showed a 1 log decrease and, when wetted, an even larger decrease. Neither high RH nor wetting impacted the ability of the fiberglass duct board or insulation to support growth. That is, the level of *Penicillium* on the samples remained essentially the same throughout the experiment.

Effect of RH on Growth for New Materials. Figure 2 shows the test results for the second series of moisture experiments. The test material was newly purchased FDL-A equilibrated to four RHs, 85, 90, 94, and 97%. The corresponding moisture content was 3% for the samples maintained at 97% RH, approximately 2% for those in the 94% RH chamber, and <2% at the remaining RHs. In the 97% RH chamber, after a brief decline in CFUs measured on day 7, the levels of *P. chrysogenum* increased steadily through day 28. The log increase from day 1 was over 2 logs and, overall, 3 logs from the low point on day 7. The samples maintained in the other three chambers, 85, 90, and 94% RH, showed an initial 1 log decrease at day 7 similar to that seen in the 97% RH chamber. No increase (or decrease) was seen in the levels of CFUs on samples maintained in the 85 and 90% RH chambers over the 6-week course of the experiment; however, a slight increase was measured on day 42 for the samples in the 94% chamber.

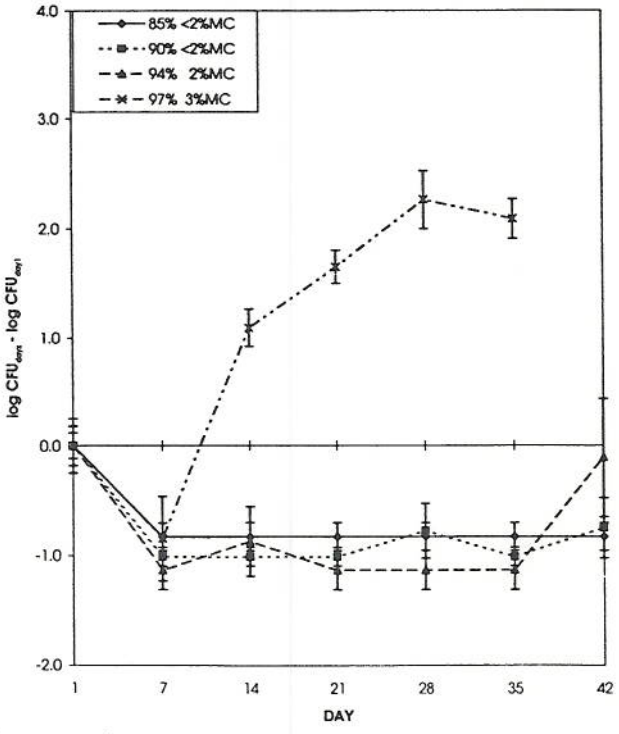

Fig. 2 Growth of *P. chrysogenum* on FDL-A at 4 RHs

These data show that, for a particular material and organism, even very slight and difficult to measure changes in duct RH (in this case, between 94% and 97%) might have important effects on the growth of the microorganism. Even slight improvements in design could have a major impact on the likelihood of microbial reservoirs being established. Near saturation conditions (97% RH) are not the norm in HVAC duct systems, but can be encountered, for instance, near condensers or near standing water.

Effect of Soil

In order to evaluate the impact of soil on the growth of *P. chrysogenum* on the newly purchased duct materials, samples of all materials were subjected to the artificial soiling procedure. Two levels of soiling were used: moderately soiled (50–100 mg dust/100 cm^2) and heavily soiled (1000–2000 mg dust/100 cm^2). These levels were selected with reference to the 1.0 mg dust/100 cm^2 definition of cleanliness given in NADCA (National Air Duct Cleaners Association) Standard 1992-01. NADCA 92-01 states that a surface may be verified as clean only if the surface is visibly clean and if the weight of the debris collected by the NADCA vacuum test does not exceed 1.0 mg dust/100 cm^2. This standard is intended only for non-porous surfaces, but its definition of the amount of soil that may remain in a "cleaned" duct provides the only quantitative benchmark available. In this study the vacuum test was not performed: soil loading was determined by weight. Therefore, moderately soiled, as targeted in this research, was 50 to 100-fold higher than the standard, while heavily soiled indicates duct materials loaded 1000 to 2000-fold NADCA 92-01. The weight of soil, calculated as shown in equation 2, on moderately soiled FDL-A samples, ranged from 22 to 136 mg/100 cm^2, while those for heavily soiled FDL-A ranged from 980 to 2384 mg/100 cm^2. Upon visual inspection, the surface of the materials defined as moderately soiled had a relatively small amount of dust evenly distributed across a surface that still clearly showed its fibrous character. The heavily soiled samples appeared to be evenly coated with dust, and few if any actual fibers were visible. The levels chosen for the moderately soiled samples were within the range of those reported in actual ducts, where amounts ranging from 68 to 182 mg/100 cm^2 have been reported (Luoma et al, 1993).

Moderately soiled. The black bars in Figure 1 present the data for the test materials that were moderately soiled, inoculated, and placed in the 97% RH chambers for 42 days. For all materials, the moderate soiling treatment caused significant increases in fungal growth. Although new, FDL-A was able to support the growth of

the test organism under conditions of both 97% RH and wetting; soiling enhanced the microbial amplification. As shown earlier, *P. chrysogenum* inoculated on new FDL-B demonstrated a decrease in log CFU at 97% RH and an even larger decrease upon wetting. However, under conditions of 97% RH and moderate soiling, a 3 log increase from day 1 levels was attained after 42 days. FDL-C maintained at 97% RH demonstrated no growth of *P. chrysogenum* in 42 days. However, when moderately soiled, FDL- C showed a 2 log increase in CFUs, comparable to that seen when wetted. Both the fiberglass duct board and insulation also demonstrated significant increases in log CFU when moderately soiled.

Heavily soiled. Similar experiments were performed on FDL-A that had been heavily soiled. Previous experiments (see Figure 2) at 85, 90, 94, and 97% RH, had shown that only the samples in the 97% RH chamber had demonstrated a sizable increase in log CFUs over 5 weeks. (The slight increase detected on those in the 94% RH chamber from the numbers of CFUs at 5 weeks to the level seen at 6 weeks may have been significant.) Since growth at 97% RH had already been demonstrated for moderately soiled samples, the heavily soiled test was only conducted on FDL-A placed in the 85, 90, or 94% RH chambers for 6 weeks. As shown in Figure 3, when heavily soiled, all samples at all RHs were able to support the growth of the *P. chrysogenum*. The samples in the 90 and 94% RH chambers, after a slight decrease on day 7, increased by 2 logs by day 14 and 3 logs by day 35. Even the samples placed in the 85% RH chamber demonstrated a 1 log increase in *P. chrysogenum* spores between the 4th and 6th week.

In summary, all the materials tested, when artificially soiled to the moderately soiled level and maintained at 97% RH for 6 weeks, demonstrated at least a 1 log increase over new, clean materials similarly maintained at 97% RH. *P. chrysogenum* on samples of FDL-A, which had previously demonstrated little or no notable increase at RHs of 85, 90, and 94% after 6 weeks (Figure 2), demonstrated rapid growth at 90 and 94% RH when heavily soiled. Although the *Penicillium* on heavily soiled FDL-A demonstrated an increased lag period at 85% RH, by the end of the 6-week experiment amplification was seen on those samples too.

Growth on Used Duct Materials

Used materials from buildings with no known biocontamination problems were collected to evaluate the impact of use on the ability of the materials to support the growth of *P. chrysogenum*. Efforts were made to select materials that matched the ones newly purchased for the project. Experienced local contractors identified those that, to the best of their knowledge, were the same brands. All used materials were estimated to be between 5 and 10 years old. None of the materials appeared exceptionally soiled or frayed, although all were obviously used.

Figure 4 shows the log CFU change over the 6-week experiment on samples maintained at 97% RH. At the

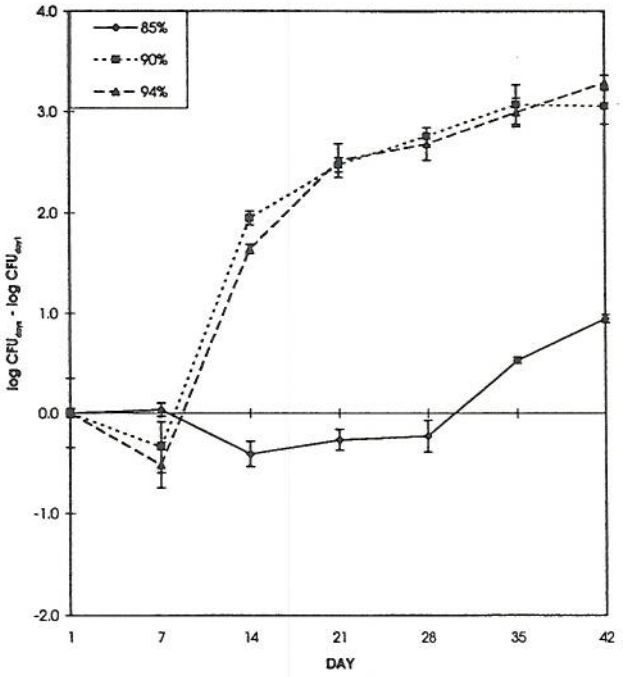

Fig. 3 Growth of *P. chrysogenum* on heavily soiled FDL-A at 85, 90, and 94% RH

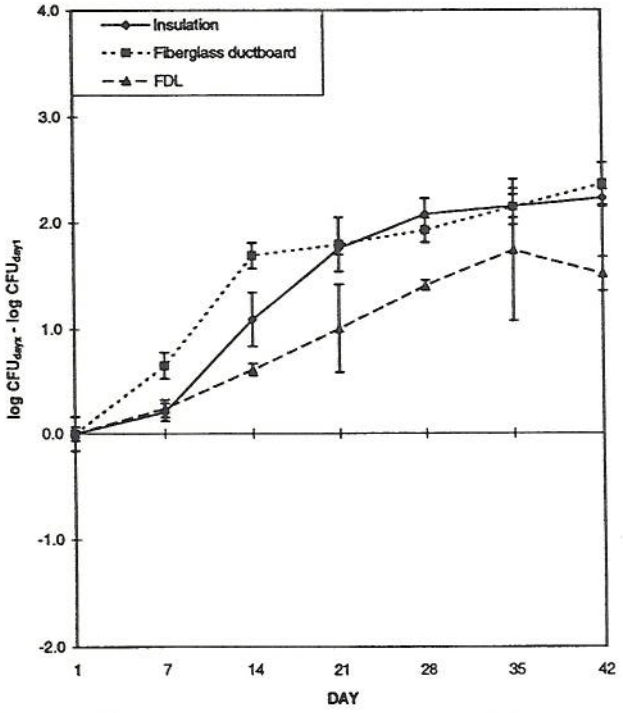

Fig. 4 Growth of *P. chrysogenum* on used materials at 97% RH

end of the experiment all materials were able to support the growth of *P. chrysogenum*. The CFUs had increased 2 logs on the fiberglass duct board and insulation, and 1.5 logs on fiberglass duct liner.

Effect of Temperature

The air temperature and RH in HVAC systems vary over a wide range. The air passes through coils where it may be either heated or cooled. In the cooling season the air temperature and resulting material surface temperature may be quite low. Temperatures as low as 12°C in a cold deck are not unusual in a typical chilled water or direct expansion type cooling system (Colen, 1990). In addition, the humidity of the air leaving a cooling coil is near saturation.

Figure 5 shows the growth of *P. chrysogenum* at 12°C and 23°C on newly purchased FDL-A maintained in the 97% RH chamber for 5 weeks. The results at 23°C showed a slight decrease in CFUs on day 7, followed by a 2 log increase from day 7 to day 14, and finally to an increase of over 3 logs by day 28 from the low point on day 7. At 12°C, the CFUs of *P. chrysogenum* decreased steadily for the first 3 weeks. However, by day 28 the change in log CFUs from day 1 had increased over 1 log, and by day 35 over 2 logs. Between the lowest point (day 21) and the final (day 35) sampling, the CFUs had increased almost 4 logs. The results demonstrated that, while low temperatures may delay the onset of growth, they do not arrest growth. Over the full 35-day experiment, comparable log increases of CFUs were attained even though there was an extended lag time at 12°C.

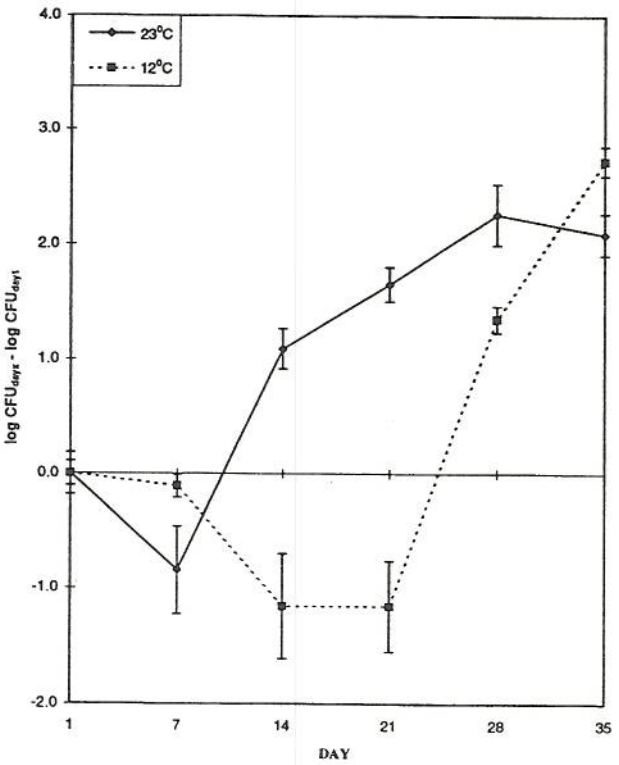

Fig. 5 Growth of *P. chrysogenum* on FDL-A at 23°C and 12°C at 97% RH

Conclusions

This study showed that both moisture and soiling were important influences on fungal growth on fiberglass duct materials. Sustained exposure to 97% RH allowed amplification of the mold on samples of at least one of the new fiberglass duct liners (FDL-A), but not on samples of the other four products. Wetting the materials increased the susceptibility to *P. chrysogenum* of at least two of the duct liners (FDL-A and FDL-C) tested, but not the other duct liner (FDL-B), the fiberglass duct board, or the insulation. These results indicate that the nutrients required for growth of *P. chrysogenum* were probably part of the material itself for FDL-A and FDL-C. While fiberglass alone is not thought capable of supporting microbial growth, apparently either the binder material or materials added during processing are sufficient in some cases. That growth did not occur under the same conditions for all samples suggests that there may be significant differences between lots of one product and/or formulations for different but very similar materials.

Artificial soiling significantly enhanced the ability of all duct materials tested to support the growth of *P. chrysogenum*. Two effects were observed: first, when FDL-A was heavily soiled, the organism was able to grow at RHs considerably lower that those needed for growth on the untreated newly purchased material; and secondly, *P. chrysogenum* could grow on moderately soiled fiberglass materials that had not supported growth before soiling. The most likely explanation is that the HVAC dust provided required nutrients that were not available without soiling. In addition, application of the soil may have increased the hygroscopicity of the materials, thereby increasing the available moisture and permitting the growth of the *P. chrysogenum*.

The effects of moisture and soiling were especially notable on the biocide-containing material, FDL-B. Decreases in numbers of CFUs, probably reflecting the death or inactivation of the *P. chrysogenum* spores, were seen when the moisture content of the new FDL-B was increased by exposure to high RH or wetting. Apparently, the biocide was able to remain in contact with the organism under these relatively clean conditions. However, when FDL-B was moderately soiled, significant growth of *P. chrysogenum* occurred. It is reasonable to hypothesize that the new substrate, formed by soiling, masked the effect of the biocide or prevented contact of

the spores with the biocide, and provided favorable conditions for fungal growth.

The data from the current studies showed that a very low moisture content (2–3%) and soil loading (50–100 mg/100 cm^2) were sufficient for amplification of *P. chrysogenum* within 6 weeks. While this was a limited study and only one batch of each material was tested, representative samples of those commonly found in the market place were utilized. Many of these materials or materials similar in composition have been in use for a number of years. As was seen by the results for the used materials tested, in order to prevent or minimize the potential for fungal growth on fiberglass materials, they must be kept clean and dry. Even small amounts of soil or moisture can permit or enhance the amplification of fungi. While lower temperature (12°C) might delay the initial lag period for *P. chrysogenum*, growth was not arrested. In fact, the data showed that, after the initial delay, *P. chrysogenum* at 12°C eventually reached a similar level of growth to that at 23°C by the end of the 35-day test. Therefore, the low temperatures expected in the cold decks of HVAC systems probably will not inhibit or limit the growth of *P. chrysogenum* on fiberglass materials.

Dust enters a HVAC system through either the return air ducts or infiltration paths (leaks and faulty joints) on the suction side of the system. Reducing the infiltration of dusty air is therefore a biocontaminant issue as well as energy and equipment maintenance issues. Similarly, these data support the use of more and better filtration of the air entering a HVAC system. While the results of this study apply directly only to fiberglass duct materials, they suggest that dust accumulation and/or high humidity needs to be properly controlled on any HVAC duct surface, regardless of material, to prevent the growth of *P. chrysogenum*. Finally, because some soil unavoidably enters HVAC systems, frequent inspection and possibly cleaning of interior surfaces that are exposed to elevated humidities or other sources of moisture are desirable.

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