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Effect of heating-ventilation-air conditioning system sanitation on airborne fungal populations in residential environments

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Commercial air duct sanitation services are advertised to the public as being effective in reducing indoor aeroallergen levels despite the absence of published supporting data. Eight residential heat-ventilation-air conditioning (HVAC) systems in six homes and seven HVAC systems in five homes in winter and summer, respectively, were sampled to determine fungal colony forming units (CFUs) prior to and after an HVAC sanitation procedure was performed by a local company. Two houses in which no sanitation procedure was performed served as controls in each study phase. Two sample sets were obtained at each HVAC system prior to cleaning in order to determine baseline CFU levels. The test HVAC systems were then cleaned, and the HVAC systems allowed to operate as desired by the residents. Posttreatment sampling was performed 48 hours and then weekly after cleaning for 8 weeks. The HVAC systems were analyzed by exposing sterile 2% malt extract media plates at a 90-degree angle to the air flow at the air supply and air return vents. The baseline CFUs were similar in the control and study houses. Eight weeks after sanitation, the study houses demonstrated an overall CFU reduction of 92% during winter and 84% during summer. No reduction in CFU values was observed over the 8-week study period for the houses selected as controls. Further, HVAC sanitation appeared to reduce the number of fungal colonies entering and leaving the HVAC system, suggesting that the HVAC contained a significant percentage of the total fungal load in these homes. These data suggest that HVAC sanitation may be an effective tool in reducing airborne fungal populations in residential environments.

INTRODUCTION

One of the fundamental methods of treating allergic disease is the avoidance of offending allergens. This is often difficult to achieve with indoor aeroallergens, particularly those with microscopic sources such as house dust mites and fungal spores. The relationship of indoor fungal allergens to allergic rhinitis and asthma has been a source of study for many years.

Respiratory disease can be caused by humidifying devices contaminated with fungi, and contamination of air conditioning systems has been linked to hypersensitivity pneumonitis.[1] Collections of the mycoflora from air conditioned as well as evaporatively cooled homes have been shown to have increased populations of Aspergillus and Penicillium species.[2,3] The heating-ventilation-air conditioning (HVAC) systems of both residential and commercial buildings have been shown to be conducive environments for fungal aeroallergen proliferation and amplification. Other indoor sources, such as wet carpets, provide suitable substrates for fungal colonization and amplification as well.[4-6]

Many devices purported to "clean" air are currently being marketed to the public. Examples of these devices are free-standing electrostatic air cleaners, negative ionizers, electrostatic precipitators, air conditioning filters, gas and odor absorbent and HEPA filters. Indeed, an air conditioner alone acts as an efficient air cleaning device.[7] Not all of these methods have been found to be effective[8-10] and the authors have found no literature regarding the efficacy of HVAC sanitation, or "air duct cleaning," as it is commonly known.

The authors evaluated the commercial HVAC sanitation procedure of a local air duct cleaning company to determine its ability to decrease residential fungal populations. The protocol allowed for sampling prior to the HVAC sanitation procedure to establish a population baseline for each test house and eight weeks of post-treatment sampling to determine the fungal populations' response with time.

The study was divided into two phases. The first phase evaluated the HVAC sanitation procedure during the winter months when the systems were operating in the heating mode. There were a total of six houses tested with two of the houses serving as the control group. Fungal populations were determined by counting colony-forming units (CFUs) growing on culture media plates exposed to air flows entering and leaving the HVAC system. The second

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phase evaluated the HVAC sanitation procedure during the summer months when the systems were operating in the cooling mode including an additional quantitative analytical method not used in the first phase.

MATERIALS AND METHODS

Phase I—Winter

The houses studied were identified through the offices of three local board-certified allergists. Patients who exhibited chronic symptoms of allergic rhinitis and who had one or more positive epicutaneous skin tests to indoor molds were informed of the study. The parameters of the study were explained to each potential participant, and those who agreed to participate in the study did so on a voluntary basis. Table 1 describes the houses used in phase I of the study. Two of the houses had two HVAC systems. All were in the Fort Worth metropolitan area. The control houses were selected by the authors (R.A.G., L.D.R.), one relatively new house and one older house.

Each house was initially studied two times prior to the commercial HVAC sanitation procedure. After HVAC sanitation, the houses were sampled at 48 hours, then weekly for a total of 8 weeks. The control houses were sampled on the same schedule.

Table 1. Description of Houses Studied

House	Sq. Ft.	Age, yr	Construction	HVAC Comments
Phase I—winter				
1	1900	30	Brick and wood frame	Light fungal growth on coils and several registers
2	2300	50	Brick	Unit A: moderate fungal growth on registers Unit B: no visible fungal growth
3	4600	60	Brick	Unit A: heavy fungal growth on fan, coils, and plenum Unit B: heavy fungal growth on fan, coils, and plenum
4	2100	32	Brick and wood frame	Heavy fungal growth on coils, plenum and registers
5 Control	2200	5	Brick	Light fungal growth in drip pan
6 Control	3800	58	Brick	Heavy fungal growth on coils and plenum
Phase II—summer				
1	2200	15	Brick and wood frame	Unit A: light fungal growth on coils and plenum Unit B: light fungal growth on coils and plenum
2	1800	16	Brick	Heavy fungal growth on registers and adjacent ceiling texture
3	2200	12	Brick	Heavy fungal growth on fan, coils, plenum and registers
4	1700	23	Brick	Heavy fungal growth on coils, plenum and registers
5 Control	1500	8	Brick	Light fungal growth in drip pan
6 Control	1600	16	Brick	Heavy fungal growth on coils, registers, and plenum

The sampling procedure was performed by exposing standard 110 x 15 mm sterile plates containing 20 mL 2% malt extract agar. This media appears to be the most suitable for isolation of indoor airborne fungi.[11] Exposed culture plates were incubated at 25 °C under natural day/night light cycles. Colony forming units (CFUs) were determined at three, five, and seven days after exposure. The plates were discarded after 10 days. All mature sporulating isolates were identified using standard light microscopic and mycologic techniques to enumerate and identify the taxa of viable fungal aeroallergens inhabiting or being disseminated by the HVAC system. A dichotomous taxonomic key was used to verify the identity of fungal genera isolated.[12]

Air velocity at the sample locations (supply and return vents) was measured with a Taylor (Syborn Corp) vane anemometer. Air velocity was recorded in feet per minute for a 1-minute period. Air velocity measurements were taken in triplicate for each sample location. The fan for the HVAC system was turned to "manual on" during all measurements and/or sampling. Samples were obtained during the winter months while the heating units were in use, with the windows in the house closed.

The HVAC system was sampled by exposing plates directly in the stream of air either exiting (supply) or entering (return) the HVAC system. Culture plates were oriented so that the moving air stream impacted the plates at a 90-degree angle relative to the media surface. Culture plates were exposed for exactly ten minutes. This procedure allowed for the direct impaction of fungal propagules exiting or entering the HVAC system.

Relative humidity was measured with a Syborn wet-dry bulb psychro-

meter with each sample event in phase I.

Phase II—Summer

Six separate houses were identified as previously described for the phase I study. Table 1 describes the houses used in phase II of the study. One test house had two HVAC systems. All were in the Fort Worth metropolitan area. The control houses were selected by the authors (R.A.G., L.D.R.). The same sampling protocol as described above was followed for the study houses. The control houses were sampled at random times during the 10-week test period.

An additional analytical technique used for phase II was quantitative bioaerosol collections. The quantitative bioaerosol data was obtained using the Burkard Personal Spore Trap. This instrument was calibrated at an air flow rate of 10 L/min prior to sampling. Air samples were collected for either a 5-minute or a 2-minute period. The Burkard was placed in the master bedroom on the nightstand near the head of the bed in each home. In homes with two HVAC units, the Burkard was placed on the kitchen counter nearest the refrigerator. The Burkard was placed in positions that were hopefully representative of the breathing zone, and was not oriented towards supply or return vents located in the room being tested. The samples were analyzed by staining the trace with augmented Calberla's solution and quantified by light microscopy. Numerical values were expressed as total fungal propagules/m^3 (includes hyphal fragments as well as discrete spores).

HVAC Sanitation Protocol

The HVAC sanitation procedure was performed by Abbey Road Clean-Aire of Forth Worth, Texas. The vent registers in the home were first removed, taken outdoors, and cleaned with a 0.25% glutaraldehyde solution. A HEPA-filtered vacuum was used to clean each outlet, the return air ducts, and the HVAC unit. The vent openings in each room and the plenum area above the HVAC unit were cleaned with the glutaraldehyde solution, rinsed with water, and wiped clean. A vinyl-copolymer was fogged into the duct runs from the unit and into each air duct outlet using airless spray equipment. The evaporative cooling coils were disassembled, sprayed with the glutaraldehyde solution, brushed thoroughly, rinsed with water, and reassembled. Any access ports made in the air duct system were then secured and the vent openings replaced. A permanent, washable electrostatic air filter was installed in each system. Residents of all houses involved in the study cleaned their houses as usual during the study.

Skin Testing Protocol

Two hundred pediatric and adult patients who underwent allergy skin testing to a panel of regionally significant allergens were tested to a selection of mold extracts as part of their clinical evaluation. All of the patients tested were under the care of one of four board-certified allergists and were being evaluated for allergic rhinitis and/or asthma. None of the patients had received antihistamines or other medications such as antidepressants known to suppress skin histamine wheal and flare response at least 72 hours prior to testing, and no patient had taken astemizole in the 6 weeks prior to testing. No patient was receiving allergy immunotherapy at the time of testing. Skin testing was performed using the commercially available Multi-Test (Lincoln Diagnostics, Inc., Decatur, IL) method, with glycerinated extracts (1:10 concentration) applied to the patient's forearm for 15 minutes. Extracts were purchased from Center Laboratories (Port Washington, NY). Skin test results were read as follows: negative: no wheal, 1+: erythema only, 2+: wheal less than 4 mm in diameter, 3+: wheal between 4 and 8 mm, 4+: wheal greater than 8 mm with or without pseudopod formation.

RESULTS

Phase I—winter

Initial baseline sampling of the test houses and the control houses yielded results that indicated large fluctuations in the absolute value of fungal isolates observed during each discrete sample cycle (Table 2). This observation is common to other indoor residential fungal population studies.[8] Colony forming unit values obtained ranged from 2 to 23. While there was variation in the CFU value observed during each collection, the trend of high or low CFU values for each sample site was consistent. The standard deviation calculated for the baseline averages in the test houses was comparable to the standard deviation calculated for the control houses. This is important to note since the study houses had a much larger sample data base for determination of the standard deviation.

Table 3 shows the average CFU values obtained for the test houses during the 8 weeks of posttreatment sampling and the calculated percentage decrease in fungal CFUs for phase I. Each HVAC unit that underwent the sanitation procedure yielded a significant decrease ($P < .025$ by the Student's t test) in the number of CFUs obtained over the 8-week posttreatment sampling period. The average decrease in CFUs was 91.6%. The control houses both experienced a 13% increase during the same 8-week sampling period. At no time during the study were CFU values found in the control houses as low as the observed values for the posttreatment samples in the study houses.

The air flow of each HVAC system was measured with a vane anemometer to determine the feet per minute of air flow at the point of sample collection. This was done in order to provide data for comparing the amount of exposure of air in cubic meter per min (m^3/min) as

Table 2. Phase I—Total CFUs for Study and Control Houses (winter)*

	Baseline		Posttreatment								
			48 hr	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk	8 wk
Study Homes											
1-S†	11	8	2	0	—	0	1	0	0	0	1
1-R	8	7	2	2	—	1	0	1	0	1	0
2A-S	12	9	1	1	—	2	2	1	1	0	1
2A-R	10	13	0	0	—	0	0	0	0	1	1
2B-S	2	7	0	1	—	0	0	1	0	0	1
2B-R	5	5	0	0	—	0	1	0	1	0	1
3A-S	23	20	1	—	2	1	—	—	1	1	0
3A-R	12	9	3	—	1	1	—	—	2	0	0
3B-S	6	7	0	—	0	0	—	—	1	4	3
3B-R	5	8	0	—	1	0	—	—	1	1	1
4-S	18	13	0	0	—	0	0	0	0	0	0
4-R	6	5	3	2	—	1	2	1	0	2	1
Control houses											
C1-S	6	9	5	11	—	8	—	10	14	8	6
C1-R	8	5	7	8	—	6	—	5	4	8	3
C2-S	15	9	8	8	—	17	—	11	12	8	21
C2-R	8	6	6	3	—	11	—	10	13	14	19

* Total CFUs per culture plate exposed perpendicular to air flow from HVAC vent for ten minutes.
† Abbreviations: S = supply vent, R = return vent, and — = not sampled.

(Fig. 1; r = .0015). Colony forming unit numbers after HVAC sanitation were uniformly quite low, and were not used to correlate with humidity data.

Phase II—summer

Table 5 presents the total CFU values for both the test and control homes for phase II. Initial baseline sampling of the study houses and the control houses yielded results that also indicated large fluctuations in the absolute value of fungal isolates observed during each discrete sampling cycle. The homes in the treatment group had a mean of 484 ± 60 CFUs for the initial baseline sampling. The homes in the control group had a mean of 132 ± 153 CFUs in the initial baseline sampling.

Table 6 shows the average CFU values obtained for the test homes during the pretreatment, 8 weeks of posttreatment, and the calculated percentage decrease in fungal CFUs. Each HVAC unit that underwent the sanitation procedure yielded a significant decrease ($P < .025$ by the

Table 3. Pretreatment/Posttreatment, and Percent Change in Average CFUs with HVAC Sanitation (winter)

Study Houses	Base-line	Post-treatment	% Change
1	17	1.4	−92*
2A	22	1.4	−95*
2B	9.5	0.8	−95*
3A	32	1.8	−94*
3B	13	1.9	−86*
4	21	1.5	−93*
		Average	−92
Controls	7	8	+13
	10	11.5	+13

* $P < .025$.

opposed to exposure with time alone. The air flow values obtained (Table 4) for the test group and control group averaged 0.86 ± 0.17 m^3/min exposure to the culture plate at the supply vent. The air flow values obtained for the study group and control group averaged 0.43 ± 0.13 m^3/min at the return air vent. These data indicate that there was not a significant difference in the amount of air impacting the culture plates for either the study or control houses.

Relative humidity was less than 50% in most of the houses tested, and was between 50% and 60% in a minority of samples. There was no correlation between CFUs obtained prior to HVAC sanitation or in control houses and relative humidity

Table 4. Average Ft/Min and CFM* for Phase I (winter) Test and Control Houses

House	Test 1	Test 2 ft/min	Test 3	Average ft/min	Average m^3/min*
Supply vents					
1	578	577	595	583	0.96
2A	453	465	457	458	0.76
2B	411	351	400	387	0.64
3A	445	451	458	451	0.74
3B	695	683	642	673	1.11
4	588	592	580	587	0.97
5	610	615	598	608	1.00
6	429	433	421	428	0.71
				Average SD	±0.86
					±0.17
Return vents					
1	289	288	282	286	0.47
2A	260	280	266	269	0.44
2B	149	148	154	150	0.25
3A	281	270	282	278	0.46
3B	224	227	232	228	0.38
4	365	358	362	362	0.60
5	350	360	362	357	0.59
6	168	178	167	171	0.28
				Average SD	0.43
					0.13

* m^3/min was calculated based on the air velocity at the vent and the surface area (0.0054 m^2) of the culture plate exposed to the air stream.

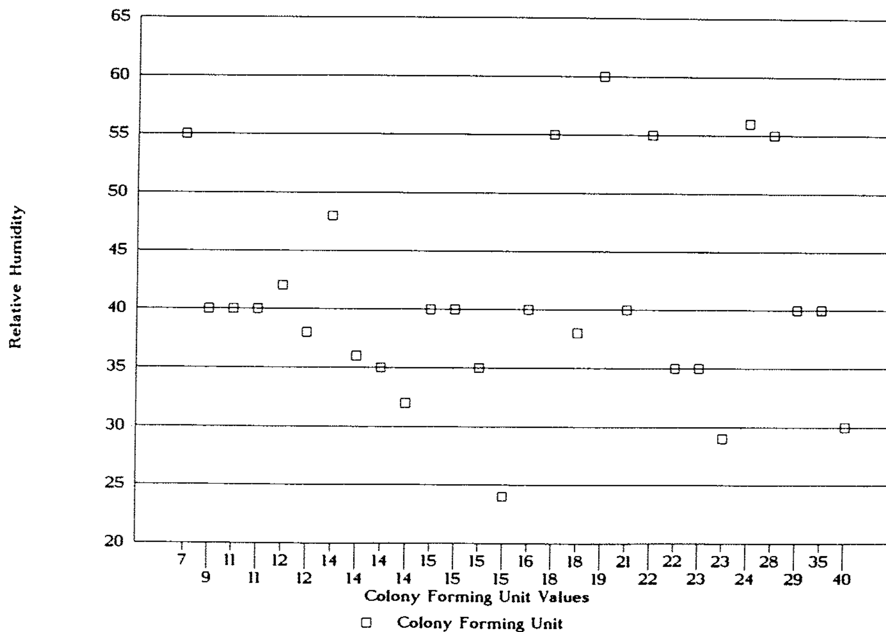

Figure 1. Correlation of CFU values obtained in non-sanitized homes with relative humidity (%), $r = .0015$.

Table 5. Phase II—Total CFUs for Study and Control Houses (summer)*

	Baseline		Posttreatment (Total CFUs)							
			1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk	8 wk
Study homes										
1A-S†	12	9	0	0	0	2	0	0	1	1
1A-R	4	4	0	0	0	0	1	3	0	0
1B-S	6	14	1	0	0	2	0	1	0	0
1B-R	3	7	2	3	1	6	1	1	2	0
2-S	17	21	3	—	—	3	6	—	6	3
2-R	16	52	7	—	—	1	4	—	3	2
3-S	279	118	1	1	10	—	3	3	3	5
3-R	484	210	0	4	1	—	2	1	2	0
4-S	387	125	0	1	6	—	4	5	5	2
4-R	500	151	0	6	4	—	5	2	5	3
Control houses										
C1-S	10	6	—	9	15	—	—	9	9	18
C1-R	5	3	—	11	11	—	—	8	4	7
C2-S	55	60	—	76	—	—	—	97	39	82
C2-R	51	74	—	150	—	—	—	39	77	53

* Total CFUs per culture plate exposed perpendicular to air flow from HVAC vent for ten minutes.
† S = supply vent, R = return vent, and — = not sampled.

Student's t test) in the number of CFUs obtained over the 8-week posttreatment sampling period. The average decrease in CFUs was 84%. The control houses experienced a 66% and 24% increase, respectively, during the same 8-week sampling period. At no time during the study were CFU values found in the control homes as low as the observed values for the posttreatment samples in the study houses.

Table 6. Pretreatment/Posttreatment, and Percent Change in Average CFUs with HVAC Sanitation (summer)

Study Houses	Baseline	Posttreatment	% Change
A1	7	1	−87*
1B	8	3	−69*
2	27	8	−72*
3	273	5	−98*
4	291	7	−98*
		Average	−84
Controls	6	10	+66
	60	79	+24

* $P < .025$.

Total air volume impacting the culture plates was determined as previously described in phase I. Again, no significant difference between test and control homes was identified.

Burkard Spore Trap Results

Quantitative bioaerosol measurements using the Burkard Personal Spore Trap (Tables 7 and 8) also demonstrated similar reductions in the airborne mycoflora. Reductions in total fungal propagules ranged from 63% to 91% in homes undergoing HVAC sanitation, as compared with increases of 20% and 3% in the control houses over an 8-week sampling period.

Identification of Fungal Genera

Penicillium was by far the fungal genus most commonly identified (Table 9), followed by Alternaria, Cladosporium, Curvularia, Aureobasidium, and Aspergillus. Nineteen different genera were isolated during both phases of the study.

Skin Testing

Of 200 patients tested, 27 sets of skin tests were discarded because of a histamine reaction less than 4+, a saline reaction greater than 0, or no positive allergen responses (ie, nonallergic). The results of the remaining 173 patients were tabulated (Table 10) with ragweed reactivity found in 62%, followed by a 53% rate of Alternaria reactivity. Approximately one-fourth of patients were reactive to Aspergillus, Phoma, or Cladosporium. Only 15% of pa-

Table 7. Total Spores/m^3 for Phase II Test and Control Houses Total Spores per Cubic Meter

Baseline	1-1	1-2	Posttreatment							
			1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk	8 wk
Study homes										
1A	3350	2062	312	437						437
1B	2575	2625	344	375					438	313
2	4487	7750	343						593	657
3	5375	6938	2274		2670				1849	2350
4	5175	3063							593	468
Control houses										
C1	750									938
C2	1813									1875

Table 8. Average Spores/m^3 Pretreatment/Posttreatment, and Percent Change

	Total Spores per Cubic Meter		
	Baseline	Posttreatment	% Change
Study homes			
1A	2706	313	−88*
1B	2600	368	−86*
2	6119	534	−91*
3	6156	2287	−63*
4	4119	531	−87*
		Average	−83*
Control houses			
C1	750	938	+20
C2	1813	1875	+3

* $P < .002$.

Table 9. Total Colony Forming Units—CFUs

Genus	Phase I (winter)			Phase II (summer)		
	Pre	Post	Control	Pre	Post	Control
Penicillium	89	13	26	2287	25	438
Alternaria	55	19	139	10	14	58
Cladosporium	42	14	100	35	36	440
Curvularia	22	0	0	2	7	0
Sterile Hyphae	17	4	10	7	32	9
Aureobasidium	10	2	22	5	1	0
Aspergillus	9	1	10	0	4	17
Drechslera	5	0	3	0	4	9
Phoma	3	2	9	0	2	6
Fusarium	1	0	0	0	0	0
Acremonium	0	0	0	1	0	0
Epicoccum	0	0	12	0	6	19
Geotrichum	0	2	0	0	0	0
Nigrospora	0	0	0	1	1	1
Pithomyces	0	1	3	0	0	0
Rhizopus	0	0	0	1	2	5
Stemphyllium	0	0	4	0	0	0
Torula	0	0	0	0	1	0
Verticillium	0	0	0	0	0	2
Yeast	0	4	1	2	12	0

tients had a positive reaction to Penicillium, and 18% to Drechslera. Epicoccum elicited a positive skin test in 29% of patients. In contrast, house dust mites caused reactions in over 40% of the patients tested. Bermuda grass reactivity was found in 39%, with American elm and mountain cedar eliciting reactions in 34% each.

DISCUSSION

Millions of houses, buildings, and private businesses in the US have HVAC systems. The presence of climate controls can make the indoor environment more comfortable, and aid in the elimination of outdoor pollens and molds. An HVAC system that is poorly installed or improperly maintained, however, can serve as a primary source for fungal amplification and contribute to the indoor mycoflora.

Based on previous HVAC examination, the authors have observed fungal contamination in HVAC systems mainly in the supply air plenum (the area above the unit where the ducts join), evaporative cooling coils, drip pans, and the vent outlets in each room. Little fungal growth is typically found in long duct runs.

Excess moisture in the HVAC system is the main cause of fungal contamination. This moisture may be introduced into the system in several ways:

a. improperly installed humidifiers
b. air conditioner units inappropriately sized for the square footage of the building
c. dirty evaporative cooling coils in the unit which allow less air flow and collect condensation
d. poor drainage from the drip pan

The most common problem observed in this study was improper installation of the drip pan. The drip pan is designed to carry away the condensate that is collected from the evaporative coils during air conditioner operation. If the drainage outlet is installed incorrectly or becomes clogged with debris (therefore

Table 10. Epicutaneous Skin Test Results n = 173

Allergen	% Positive*
Penicillium drysogenum, notatum	15
Aspergillus oryzae, repens, niger, terreus	23
Phoma herbarum	25
Alternaria tenuis	53
Cladosporium spahaerosperum	24
Drechslera sorokiniana	18
Epicoccum nigrum	29
Ragweed (*Ambrosia trifida* and *artemisiifola*)	62
D. farinae	47
D. pteronyssinus	42
Cynodon dactylon (Bermuda grass)	39
Ulmus americana (American elm)	34
Juniperus asheii (mountain cedar)	34

* Percentage of patients exhibiting a wheal response of >4 mm (ie, 3+ or 4+) by MultiTest technique.

inhibiting the natural gravity drainage of this collected water), an ideal environment is formed for microbial colonization and amplification. Fungal growth is often observed on the coils, the supply and return plenums, and the wood supports for the central blower unit after the overflow of a drip pan. A properly installed and maintained HVAC system, especially when coupled with high efficiency filtration, should contribute very little to the total airborne fungal content.

Of the test houses evaluated in this study, the authors considered phase II units 2A, 3A, 3B, and 4 to have major fungal contamination. House 6, a control house, also had significant fungal contamination. The remaining houses yielded CFU values that were considered typical for this area. Significant reductions in fungal counts were obtained by the commercial HVAC procedure on all the study houses.

While the cleaning, vacuuming, and disinfection of the contaminated system is important, the installation of a high efficiency filter may be an important step in the procedure. Since the filter is installed at the return air inlet (prior to the blower and evaporative coils), fungal spores may be prevented from entering the HVAC system. A return air filter, therefore, cannot prevent an already contaminated system from introducing fungal propagules into the residence. If the HVAC system is the site of significant fungal growth, the installation of a filter of any kind is of little or no practical value without cleaning and disinfecting the system first.

This study has shown that HVAC sanitation produces a significant reduction in fungal CFUs over an 8-week period. No trend of increasing in CFUs were indicated at the end of the 8-week period. It is currently not known how long it would take any of these HVAC systems to become contaminated with fungal growth again. The authors were unable to perform further sampling in a majority of these homes because of families moving out of these homes or undertaking other activities (such as remodeling) which might affect CFU numbers.

Many of the CFU values obtained during phase II (summer) of the study were dramatically higher than those obtained during phase I (winter). There may be two possible reasons for this observed difference. First, during the summer months, the HVAC systems were operating in a cooling mode which results in a high moisture content due to the action of the evaporative coils. This high moisture content within the system may translate directly into a greater potential for fungal amplification. Second, two of the houses that were investigated in the phase II study had a much greater degree of initial contamination than the houses investigated in phase I (winter).

Concurrent outdoor analysis of fungal genera was not performed. If a single indoor fungal analysis is performed using passive methods, knowing the fungal content of the outdoor air using a similar methodology can be helpful in interpretation of the indoor data. We did not utilize concurrent outdoor data because of concerns about the appropriateness of comparing CFUs obtained by direct airstream impaction from an HVAC vent with CFUs obtained by passive methods. Control data was instead obtained from homes not undergoing HVAC sanitation so that similar methodology could be employed.

There appears to be a relatively low collection efficiency when total air volumes sampled are compared with volumetric methods such as Andersen sampling. Table 4 reports a 10-minute sample collection period to have average sample volumes of 8.6 m^3 and 4.3 m^3 for the supply and return vents, respectively. An Andersen sample collection period of ten minutes would yield a total sample volume of 0.283 m^3 (at a flow rate of 28.3 L/min). If one colony forming unit was detected for each of the three total sample volumes, the final bioaerosol concentration would be:

Supply vent = 0.12 CFU/m^3 (1 CFU/8.6 m^3)
Return vent = 0.23 CFU/m^3 (1 CFU/4.3 m^3)
Andersen = 3.50 CFU/m^3 (1 CFU/0.283 m^3)

When final bioaerosol concentrations for the HVAC sampling method are compared with Andersen sampling there is an apparent "loss" of collection efficiency of 30x at the supply vent and 15x at the return vent. If, however, one assumes both methods to be "accurate" and reflect the "true" bioaerosol concentration at the time of

sampling, then this "loss" of efficiency is actually on a difference in calculated detection limits based on total air volumes sampled. In other words, if the Andersen sampler's flow rate was increased so that in ten minutes a total of 8.6 m^3 of air was sampled and only one CFU detected, then the final bioaerosol concentration would be 0.12 CFU/m^3.

The two sampling methods cannot be directly compared, at this time, due to their inherent differences. Andersen sampling is designed to quantify the mycoflora of ambient air. The HVAC sampling method was designed to gain empirical information regarding the mycoflora of the airstream exiting and/or entering an HVAC system. The HVAC sampling method may serve as an excellent cost effective screening tool for residential environments. A correlation of the separate sampling methods will only be established by additional research. Currently, studies are being conducted to determine if there is a correlation of the HVAC sampling method relative to Andersen sampling.

No attempt was made to evaluate the clinical status of the persons living in the homes studied. Fungal allergens are a well-recognized cause of allergic rhinitis and asthma, although outdoor fungi, such as Alternaria and Cladosporium species, have been studied more extensively than have indoor molds. Penicillium and Aspergillus species are generally accepted to be indoor molds capable of eliciting allergic rhinitis and/or asthma symptoms in sensitive individuals, but other indoor mold genera are not as well characterized.

In our population of North Texas residents with allergic disease and/or asthma, the prevalence of skin test reactivity to Penicillium and Aspergillus species (15% and 23%, respectively) was slightly less than that seen to Phoma species presumably like those isolated from HVAC systems (25%). Penicillium was by far the most common indoor mold collected in both the winter and summer phases of this study.

The study of mold allergy has been complicated by the complexity of mold-related allergens, the inconsistency of commercial mold extracts, and the presence of co-existing sensitivities. If molds found in contaminated HVAC systems contribute significantly to allergic disease in the houses' occupants, then significant reduction of colony forming units should result in an improvement of clinical symptoms.

The role of HVAC sanitation in the reduction of such outdoor mold genera such as Alternaria and Cladosporium is uncertain. It is probable that the addition of a new HVAC filter during each sanitation procedure played a major role.

No correlation was found between indoor relative humidity and numbers of CFUs found. North Texas has a temperate climate which is not especially humid, and most of the relative humidity measurements were less than 50%. Higher mold levels in HVAC systems might be found in more humid climates.

In conclusion, HVAC sanitation resulted in a significant reduction of mold CFUs entering and exiting air ducts in a small number of private residences. Quantitative studies supported this finding. This reduction persisted for an 8-week period, but the total duration of the CFU reduction is unknown. Heating-ventilation-air conditioning sanitation reduced the number of fungal populations entering and exiting the HVAC, suggesting that contaminated HVACs contribute a significant proportion of the total indoor fungal aeroallergen population. Further studies are needed to confirm these findings with other sampling methods (such as immunochemical aeroallergen quantitation), different HVAC cleaning techniques, and in other climates. Longer term studies are needed to determine the duration of benefit of HVAC sanitation. More study is also needed to determine the overall importance of indoor mold skin test reactivity in the pathogenesis of allergic rhinitis and asthma, and whether HVAC sanitation is an effective environmental control measure in the management of indoor mold allergy.

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REFERENCES

1. Banaszak EF, Thiede WH, Fink JN. Hypersensitivity pneumonitis due to contamination of an air-conditioner. N Engl J Med 1965;283:271-6.
2. Sneller MR, Pinnas JL. Comparison of airborne fungi in evaporative cooled and air-conditioned homes. Ann Allergy 1987;59:317-20.
3. Solomon WR, Burge HA, Boise JR. Exclusion of particulate allergens by window air conditioners. J Allergy Clin Immunol 1980;65:305-8.
4. Hirsch SR, Sosman JA. A one-year survey of mold growth inside twelve homes. Ann Allergy 1976;36:30-8.
5. Kodama AM, McGee R. Airborne microbial contaminants in indoor environments. Naturally ventilated and air-conditioned homes. Arch Environ Health 1986;1:306-10.
6. Burge HA, Solomon WR, Boise JR. Microbial prevalence in domestic humidifiers. Appl Environ Microbiol 1980;39:840.
7. Hirsch DJ, Hirsch SR, Kalbfleish JH. Effect of central air conditioning and meterologic factors on indoor spore counts. J Allergy Clin Immunol 1978;62:22.
8. Maloney MJ, Wray BB, DuRant RH, et al. Effect of an electronic air cleaner and negative ionizer on the population of indoor mold spores. Ann Allergy 1987;59:192-4.
9. Nelson HS, Hirsch SR, Ohman JL Jr., et al. Recommendations for the use of residential air-cleaning devices in the treatment of allergic respiratory diseases. J Allergy Clin Immunol 1988;82:661-9.
10. Robertson L, Garrison R, Miller A, et al. Effect of heated air-convection

device ("Mold Zapper") on fungal aeroallergens [Abstract]. J Allergy Clin Immunol 1991;87:182.
11. Burge HP, Solomon WR, Boise JR. Comparative merits of eight popular media in aerometric studies of fungi. J Allergy Clin Immunol 1977;60:199–203.
12. Barnett and Hunter. Illustrated genera of imperfect fungi, fourth edition. New York:MacMillan Publishing Co., 1987.

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