

Long-term use of high-efficiency vacuum cleaners and residential airborne fungal-spore exposure

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Abstract

Exposure to fungal allergens is an important contributor to allergic respiratory disease, but information on the efficacy of residential fungal allergen-avoidance in allergic-disease management is lacking. Using vacuum cleaners with high-efficiency exhaust filtration is one method recommended for reducing residential allergen exposure levels, but their use to reduce fungal-spore exposure levels has not been evaluated. To evaluate the effectiveness of high-efficiency vacuuming to control airborne fungal-spore levels, fungal bioaerosols were repeatedly assessed over the course of 10 months in homes randomly assigned to groups using either conventionally filtered (control) or high-efficiency-filtered vacuum cleaners for routine vacuum cleaning. Air samples were analyzed for three fungal-spore categories representing taxa with predominantly outdoor sources and one representing taxa that commonly have indoor sources. In a two-way analysis of variance, sampling period had a significant effect on mean levels of all fungal-spore categories. Vacuum cleaner type had a marginally significant effect on the indoor spore category, with one high-efficiency vacuum group mean (of three) significantly lower than one control mean. No effect was observed of vacuum cleaner type on outdoor spore categories. Including home-environment variables in analysis of covariance models strengthened the effect of the vacuum-type treatment on the indoor spore category, with no effect on the three outdoor spore categories. Decreased indoor spore levels vs. controls were only observed in high-efficiency vacuum groups during the last sampling period, at the end of the heating season. The results suggest that using a vacuum with high-efficiency filtered exhaust could have some modest effectiveness in controlling airborne fungal-spore exposure in homes when infiltration of outdoor air is very limited.

1. Introduction

Exposure to fungal allergens is an important contributor to allergic respiratory disease. However, there is scant information on the efficacy of residential fungal allergen-avoidance in allergic-disease management (IOM, 2000; Bush and Portnoy, 2001). Clinical trials of indoor fungal allergen avoidance have not been reported. The limited available data relate to methods for reducing infiltration of spores from outdoor air, controlling the indoor environment such that fungal growth is

not encouraged and reducing fungal exposure levels in buildings where remediation of fungal contamination of building materials has been implemented (reviewed in Bush and Portnoy, 2001). Methods for reducing indoor fungal exposure levels that have been reported in the literature include air filtration, physical removal of contaminated building materials and furnishings and surface cleaning with disinfectants such as hypochlorite or quarternary ammonium compounds. Systematic studies evaluating the efficacy of high-efficiency vacuum cleaners (e.g. that use

high-efficiency particulate air (HEPA) exhaust filtration or high-efficiency bags) in reducing indoor fungal exposures have not been reported.

Long-term studies of high-efficiency vacuuming and indoor allergens have generally focused on antigens from mammalian pets or arthropods and have used antigen levels in settled dust as a surrogate for airborne levels. Results have been mixed. Vacuuming carpeting (Adilah et al., 1997) or upholstered chairs (Custovic et al., 1998) with HEPA vacuum cleaners significantly decreased dust mite antigen concentrations in settled dust compared to baseline or untreated controls over periods of 5 or 12 weeks, respectively. Antigen concentrations in carpet returned to pre-treatment levels 5 weeks after the treatment ended (Adilah et al., 1997). Thorough vacuum cleaning, either with a vacuum cleaner containing a high-efficiency bag or with a central vacuum exhausted outdoors and combined with a central dehumidification system, kept dust mite antigen levels below 2 µg/g in settled dust from newly constructed and occupied homes in an area with a hot, humid climate for up to 12 months (Chandra et al., 2000). Dust mite antigen levels were not prevented from increasing above 2 µg/g by either dehumidification or vacuuming alone. In contrast to these studies suggesting some reduction of allergen exposure by high-efficiency vacuuming, measures of dust mite, cat and dog antigen content in settled dust from carpeting, mattresses and upholstered furniture were not consistently reduced by use of high-efficiency filtered vacuum cleaners, compared to conventional vacuums, in two randomized longitudinal studies in homes of asthmatic or allergic patients (Munir et al., 1993, 1994; Poppelwell et al., 2000). Results in both studies suggested that continual reintroduction of antigen from internal or external sources limited the reduction of residential antigen levels that could be achieved with high-efficiency vacuuming.

Long-term studies relating indoor airborne fungal exposures and use of high-efficiency vacuums in homes are lacking. Therefore, a randomized exposure study was conducted to test the hypothesis that regular use of HEPA-filtered vacuum cleaners in homes would reduce airborne fungal-spore exposure levels compared to homes using conventionally filtered vacuums. The influence of HEPA vacuuming on indoor levels of several groups of fungal spores common in the indoor environment and in outdoor background air was assessed.

2. Materials and methods

2.1. Study volunteers

Volunteers from the New York State Department of Health, and from a private research and development center were recruited to participate in the allergen-avoidance study. Volunteers agreed to use vacuum cleaning equipment provided to them for home cleaning, to vacuum at least bi-weekly, to keep a running log of home-cleaning activity during the study period, and to allow air sampling to take place in their home three or four times during the study period. An adult member of each volunteer household provided written informed consent to participate in the study. Study volunteers were free to be excused from the study at any time. One volunteer relocated during the study – after only one set of samples was collected from their home – and their data were discarded. All other volunteers completed the entire study.

Participants were recruited who did not use a high-efficiency vacuum cleaner or high-efficiency vacuum bags at the time the study began. At the time the study began, volunteers' homes were required to not be subject to water intrusions such as roof leaks or flooding and to have carpeted floors in an area of the home where occupants spent much of their non-sleeping time (e.g., the living room or family room area). Participants were not recruited or selected with respect to allergic disease status.

A total of 24 homes began the study in June of 1998. All but three of the homes at the beginning of the study were single-family structures. Two homes were apartments and one was an attached townhouse. The volunteer that did not complete the study resided in one of the apartments.

2.2. Study design

The study originally followed a randomized-factorial one-way analysis of variance (ANOVA) design with repeated observations (see Section 2.7 below). The main factor was the type of vacuum cleaning equipment used in each home and consisted of five levels: (1) a newly purchased conventional vacuum cleaner (C1; Eureka model 7642, 'Bravo II') with conventional vacuum bag; (2) the participant's own conventional vacuum cleaner with conventional vacuum bag (C2); (3) the same vacuum cleaner model as in C1 with a

high-efficiency 2-ply vacuum bag (micro-filtration); (4) a newly purchased vacuum fitted with a HEPA exhaust filter and high-efficiency 2-ply vacuum bag (HEPA1; Eureka model 4472 'Powerline limited edition') and (5) a second HEPA-filtered vacuum with high-efficiency vacuum bag (HEPA2; Eureka model 6436 'Excalibur').

Participants were randomly assigned to one of the five treatment groups and were provided with the appropriate vacuuming equipment (new vacuum-cleaner bags for group C2; new vacuums and bags for all other groups). Participants were informed that the study would examine home cleaning methods and microbiological exposure levels in homes. Participants were not explicitly told the treatment group to which they were assigned, but were unblinded as to the type of vacuuming equipment provided to them. Written guidelines were provided to all study participants prior to distribution of vacuuming equipment and the initial sampling visit in order to standardize cleaning activities by participants as much as possible.

Samples were collected from each home, in a randomized order, at three or four time points during a 10-month period from June, 1998 through April, 1999. Sampling periods included June–July, September–October and November–December, 1998 and April, 1999 (hereafter referred to as the June, September, November and April sampling periods, respectively). The original study design included collecting samples from all homes ($n = 4$ or 5 per treatment group) during each of three sampling periods, including one pre-treatment period, one post-treatment period with high ambient fungal bioaerosol levels and one period with low ambient bioaerosol levels. After recognizing that substantial background fungal bioaerosol levels were still detectable during the November/December sampling period, a fourth late-winter sampling period was added. However, samples could only be collected from a limited number of homes during this fourth period ($n = 2$ each in C1, HEPA1 and HEPA2).

2.3. Home environment survey

During the initial visit to each home an adult household member completed an interview questionnaire that obtained information on the home environment and home cleaning practices. The questionnaire obtained information on building

age, length of occupancy, presence of carpeting and upholstered furniture, type of vacuum cleaner used, other floor cleaning methods used, heating type, heat distribution, use of humidification, dehumidification and air conditioning, number of bathrooms, bathroom ventilation, historical information concerning any leaks, flooding, mold growth or moldy odors and the presence of any pets and houseplants. These results are hereafter referred to as fixed home variables.

Five of the fixed home variables that might have indicated a history of water intrusions in the home were combined into a home dampness index by summing their individual results for each participant. The five variables were: (1) any current or past roof leaks in the home? (2) any current or past plumbing leaks in the home? (3) any current or past flooding in the home? (4) any noticeable mold growth in the home? and (5) any noticeable moldy odors in the home? Each of these variables was scored as zero or one (no or yes), so the combined index could vary from zero to five.

2.4. Cleaning logs

Participants were required to record information about home-cleaning effort in a daily log. New log forms were distributed to participants, and completed logs were collected from participants every 2 weeks during the period from July 20 to December 20, 1998. Participants were asked to record for each day in each 2-week period whether any of the following cleaning tasks were done that day: (1) vacuuming in their sampling area; (2) vacuuming in any other part of the home; (3) any other floor cleaning in the home (sweeping, spot cleaning, carpet shampooing, etc.); (4) dusting in any room. They also recorded for each day whether or not windows in the home were open any time during that day. The cleaning log data were summarized for each participant over the entire 5 month period and were assumed to be representative of each home during the entire study period.

2.5. Sample collection

Air samples were collected under quiescent conditions in each home using a Burkard Personal Spore Trap sampler (Burkard Corp., UK) with a flow rate of 10 l/min. Samples were collected where occupants spent most of their waking time

(living room or family room). The sampler was located as centrally as possible within the room being sampled and between 0.35 and 0.75 m above the floor. The sampler was fitted with a standard glass microscope slide on which an approximately 2×2 cm piece of double-sided, optically clear adhesive tape was mounted. Air samples were collected for 30–60 minutes, depending on season and the expected ambient fungal-spore levels. At the end of the sampling time, slides were removed from the sampler and stored at room temperature in slide storage boxes.

After collecting each air sample, a settled-dust sample was collected by vacuuming a measured area of carpet adjacent to a wall or wall-adjacent furniture for two minutes with a commercial vacuum cleaner fitted with a non-woven polyethylene dust collection thimble (Dupont). The area was a strip 0.13 m wide (the width of the vacuum-cleaner upholstery nozzle) and approximately 1–4 m long, depending on the home. The total dust mass collected was determined and dust loading (mass/unit area) was calculated for each sample.

A home-visit form was completed while the air sample was being collected. Information recorded on the form included meteorological conditions at the time of sampling, whether windows were open, whether heat or air conditioning was operating and how recently the sampling area had been vacuumed and dusted.

2.6. Spore trap sample analysis

Spore trap slides were stained with phenosafrinin in glycerin jelly and analyzed microscopically for enumeration of fungal spores by morphological identification. All spores were identified using oil-immersion, phase-contrast optics at $1000 \times$ total magnification. A custom-written PC-based computer program was used to record each spore enumerated in each of 25 taxonomic categories.

The Burkard sampler draws air through a 1×14 mm sampling inlet and impacts the air onto the surface of the adhesive tape, producing a 1×14 mm sample trace on each slide. Preliminary analyses determined that impacted total fungal-spore densities did not differ significantly along the length of the sample trace. Therefore, transects across the entire width of the sample trace were counted at regular 0.5 or 1.0 mm intervals, starting at the trace midpoint and then at alternating

positions toward the trace ends, until at least 100 total spores were enumerated, or until 15 total transects had been counted. Counting was halted on any sample with an average spore density of less than 1 spore/transect after counting five transects (one of 74 samples). The total spores present in the entire sample trace were then estimated from the total spores counted in the transects from each sample, and the fraction of the total trace area represented by the sample's transects. Volumetric spore counts were estimated based on the estimated spore total per sample trace, the sample flow rate and the sample collection time.

Data from the original 25 taxonomic categories were pooled for subsequent statistical analysis. Four categories were initially constructed to represent taxa that exclusively or primarily derive from outdoor sources: ascomycetes, basidiomycetes, *Cladosporium* spp. and miscellaneous mitosporic fungi (other than *Cladosporium* spp., *Aspergillus*/*Penicillium* spp. and *Stachybotrys* spp.). A fifth category was chosen to represent taxa that occur outdoors but also commonly have indoor sources (*Penicillium* spp. and *Aspergillus* spp., whose spores are morphologically indistinguishable). *Stachybotrys* spp. would have also been included in the indoor-source category, but *Stachybotrys* spp. were not detected in any samples. Taxa in the miscellaneous mitosporic fungi category were detected much less frequently than the other four categories and those data were excluded from further analysis.

2.7. Data analysis

Based on normal probability plots, $\log_{10}$ -transformed spore-count data generally conformed closely to normality. Prior to $\log_{10}$ -transformation, all counts were transformed as (count + 1), so that non-detect counts would be retained on the log scale. Sampling period was initially regarded as a repeated-measures variable in the factorial analysis. However, a correlational analysis carried out prior to the main factorial analysis showed that repeated observations for all airborne fungal-spore variables (which were collected 2–3 months apart) behaved essentially as independent observations. Therefore, conventional two-way factorial ANOVA was employed to analyze the airborne fungal-spore data using vacuum group and sampling period as factors. Tukey *post-hoc* multiple

comparisons were employed for pairwise comparison of treatment-level means when significant treatment effects were found.

In order to explore the possible influence of home environment variables on the crude ANOVA results, analysis of co-variance (ANCOVA) was performed on the same data sets, using dust loading and variables from the home-environment survey, the cleaning logs and the field data forms as co-variables. The analysis focused on whether inclusion of any co-variables into the ANOVA models consistently contributed to explaining variability in airborne spore concentrations and whether inclusion of any co-variables into the models reduced within vacuum-group variability sufficiently to detect an effect of the vacuum group treatment that was not detected in the crude ANOVA.

3. Results

3.1. ANOVA results

Sampling period had a significant effect on fungal spore levels for all spore groups. Mean concentrations of ascomycetes ($F_{3,56} = 34.1$, $p < 10^{-5}$), basidiomycetes ($F_{3,56} = 19.6$, $p < 10^{-5}$), *Cladosporium* spp. ($F_{3,56} = 22.9$, $p < 10^{-5}$) and *Penicillium/Aspergillus* spp. ($F_{3,56} = 7.2$, $p = 4.0 \times 10^{-4}$) were all significantly affected by sampling period.

Mean ascomycetes, basidiomycetes and *Cladosporium* spp. concentrations decreased about 10–50-fold during the November sampling period compared to the June and September periods (Figure 1). Mean *Penicillium/Aspergillus* spp. spore concentration during the November sampling period only decreased about 2-fold compared to the June and September periods, and the difference compared to baseline (June) did not quite attain statistical significance ($p = 0.07$). The April mean spore concentrations for all the fungal groups were similar to the November means, and were significantly lower than the June sampling-period results for ascomycetes, *Cladosporium* spp. and *Penicillium/Aspergillus* spp. mean concentrations, based on the multiple comparison procedure.

The vacuuming-group treatment was marginally significant for the ascomycetes ($F_{2,56} = 3.2$, $p = 0.049$) and *Penicillium/Aspergillus* spp. ($F_{3,56} = 2.8$, $p = 0.046$) spore categories. There were no significant pairwise comparisons between high-efficiency groups and control groups for ascomycetes. The HEPA group means spanned the control means for this category, with HEPA1 significantly greater than HEPA2. The mean *Penicillium/Aspergillus* spp. spore concentration for the HEPA2 group was significantly less than the mean concentration for the C1 group. No other pairwise comparisons were significant for this fungal-spore group.

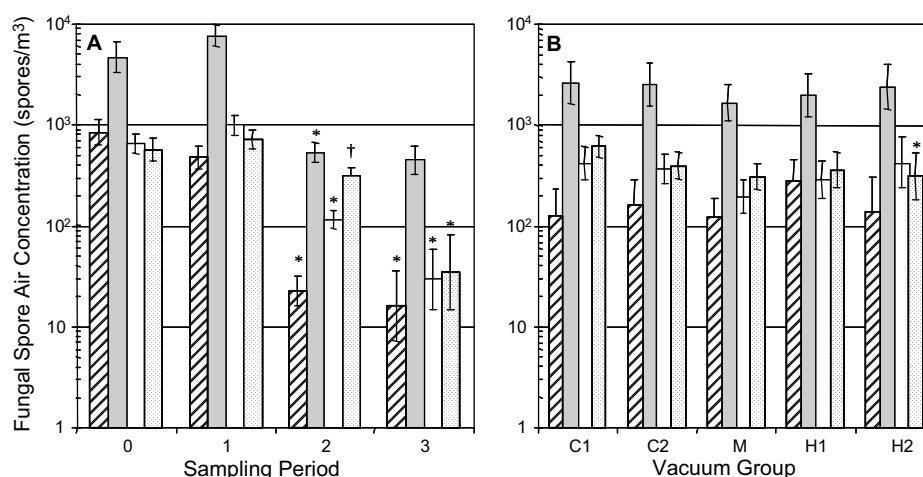


Figure 1. Fungal-spore air concentrations related to main study treatments. (▨) Ascomycetes; (■) Basidiomycetes; (□) *Cladosporium* spp.; (▤) *Penicillium/Aspergillus* spp. Geo. means $\pm$ geo. SEM. (A) Concentrations stratified by sampling period; * $p < 0.05$ by pairwise comparison vs. June/July (pre-treatment) sampling period; † $p = 0.07$ by pairwise comparison vs. June/July (pre-treatment) sampling period. (B) Concentrations stratified by vacuuming group; C1, C2 = controls; M = microfiltration bags; H1, H2 = HEPA groups; * $p < 0.05$ by pairwise comparison vs. at least one control group.

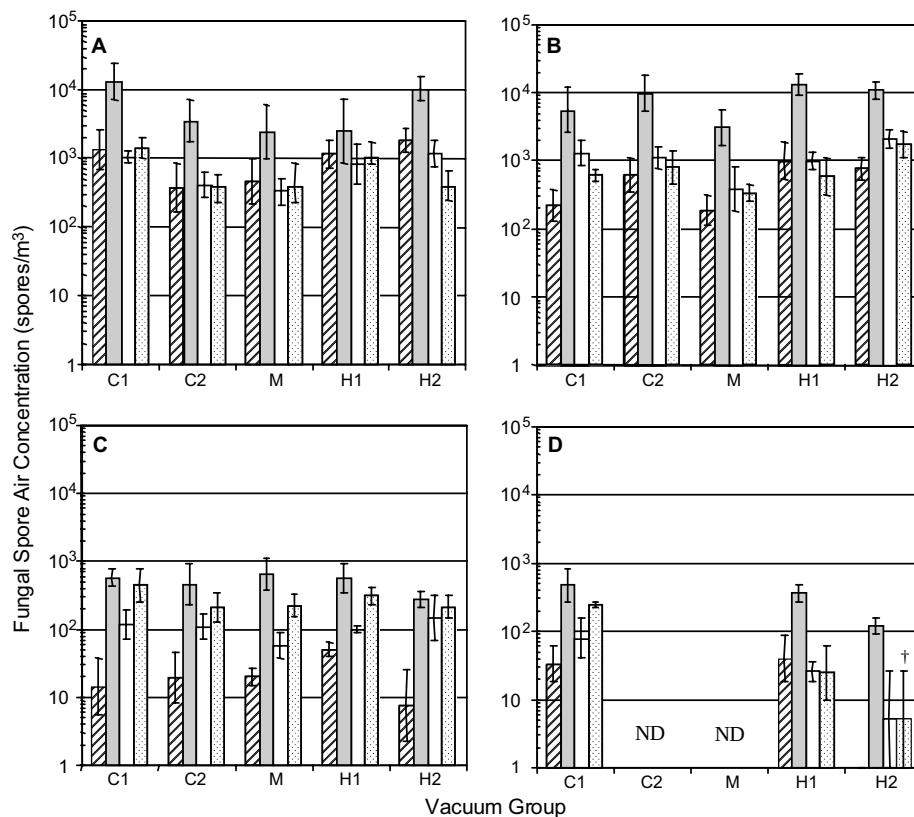


Figure 2. Fungal-spore air concentrations stratified by vacuuming group and sampling periods. (▨) Ascomycetes; (□) Basidiomycetes; (▤) *Cladosporium* spp.; (▥) *Penicillium/Aspergillus* spp. C1, C2 = controls; M = microfiltration bags; H1, H2 = HEPA groups. Geo. means $\pm$ geo. SEM. $^{\dagger}p = 0.06$ by pairwise comparison vs. C1. ND = not done. (A) June/July sampling period (pre-treatment); (B) September/October sampling period; (C) November/December sampling period; (D) April sampling period.

Vacuuming group by sampling period interaction terms in the ANOVAs were not statistically significant at the $\alpha = 0.05$ level for any of the fungal-spore groups. There was a trend toward lower mean levels in the HEPA groups compared to C1 during April for *Cladosporium* spp. and *Penicillium/Aspergillus* spp. (Figure 2). The *post-hoc* comparison between the *Penicillium/Aspergillus* HEPA2 group and the C1 group in the April sampling period was the only one to approach significance ($p = 0.06$). Mean spore concentrations for both HEPA groups during April tended to be lower than other vacuum group – sampling period combinations for the *Penicillium/Aspergillus* group (Figure 2).

3.2. ANCOVA results

Consistent differences between the two control vacuum groups and between the two HEPA vacuum groups were not found in the crude ANOVA

results. Therefore, all subsequent analyses were performed with data combined to form three vacuuming groups – controls, HEPA and microfiltration bag. To examine the relationship between vacuuming group, sampling period and airborne spore concentration further, ANCOVA was employed to reanalyze the four fungal categories using 23 different variables as potential covariates.

Most covariates included in the ANCOVA models did not contribute significantly to variation in airborne fungal-spore concentration (Table 1). Indoor and outdoor relative humidity at the time of sampling contributed significantly to models of all four fungal-spore categories, except that outdoor relative humidity was not significant for *Cladosporium* spp. Having any open windows at the time of sampling was a significant covariate for ascomycetes, basidiomycetes and *Cladosporium* spp. and the average frequency of days with any

Table 1. ANCOVA results – *p* values for tests of the effects of individual co-variates on fungal-spore levels

Potential co-variates	<i>Ascomycetes</i>	<i>Basidiomycetes</i>	<i>Cladosporium</i> spp.	<i>Penicillium</i> / <i>Aspergillus</i> spp.
Cleaning-log variables				
Frequency of vacuuming in sampling area	0.072	0.020*	0.013*	0.13
Frequency of any vacuuming in home	0.19	0.081	0.039*	0.13
Frequency of any floor cleaning	0.92	0.72	0.98	0.10
Frequency of any windows open	0.023*	0.0026*	0.11	0.14
Variables at sampling event				
Time since sampling area last vacuumed	0.87	0.55	0.35	0.60
Indoor relative humidity	0.0035*	< 10 ⁻⁵ *	0.0036*	0.00005*
Outdoor relative humidity	0.0027*	0.0074*	0.99	0.016*
Indoor temperature	0.089	0.17	0.33	0.67
Outdoor temperature	0.85	0.012*	0.084	0.17
Windows open	0.0011*	< 10 ⁻⁵ *	0.00022*	0.059
Carpet dust loading	0.27	0.62	0.77	0.52
Fixed home variables				
Age of home	0.23	0.16	0.13	0.14
Type of heat	0.83	0.28	0.33	0.77
Type of heat distribution	0.074	0.16	0.83	0.84
Home dampness index	0.15	0.37	0.82	0.56
Number of bathrooms	0.16	0.025*	0.065	0.26
Number of houseplants in sampling area	0.66	0.66	0.11	0.83
Use of humidifier in home	0.38	0.44	0.39	0.86
Use of dehumidifier in home	0.39	0.58	0.48	0.37
Air conditioning in home	0.13	0.086	0.40	0.058
Basement in home	0.32	0.95	0.40	0.55
Pets in home	1.0	0.87	0.83	0.23
Carpeting height	0.56	0.41	0.066	0.56

p < 0.05 (*) indicates co-variate contributed significantly to explaining variation in fungal-spore concentration in the vacuuming-group × sampling period factorial analysis.

windows open during the study was a significant covariate for airborne concentrations of ascomycete and basidiomycete spores. Whether windows were open during sampling and the average frequency of days with open windows did not significantly contribute to explaining variation in *Penicillium/Aspergillus* spp. Average vacuuming frequency, either in the sampling area or anywhere in the home, explained significant variation in mean airborne concentrations of basidiomycetes and *Cladosporium* spp. and approached significance for ascomycetes. Indoor and outdoor temperature at the time of sampling, age of home, type of main heat source, type of heat distribution system, self-reported home dampness indicators, the number of bathrooms, the number of houseplants in the sampling area, presence of a humidifier, dehumidifier or air conditioner, presence of a

basement, presence of pets, the carpet height in the sampling area and carpet dust loading in the sampling area did not consistently contribute significantly to the models as predictors of airborne fungal-spore concentration.

Controlling for variation explained by single co-variates in the ANOVA analysis failed to result in significant treatment effects for the vacuum-group treatment on mean ascomycete, basidiomycete or *Cladosporium* spp. spore concentrations (Table 2). Similarly, vacuuming-group by sampling-period interaction terms generally remained not significant for these three fungal-spore groups when single co-variates were included in the model (Table 3).

Including single co-variates in the ANOVA model for *Penicillium/Aspergillus* spp. resulted in significant vacuum-group effects in four cases

Table 2. ANCOVA results – p values for tests of the effects of individual co-variables on the significance of the vacuuming-group treatment effect

Potential co-variables	<i>Ascomycetes</i>	<i>Basidiomycetes</i>	<i>Cladosporium</i> spp.	<i>Penicillium</i> / <i>Aspergillus</i> spp.
Cleaning-log variables				
Frequency of vacuuming in sampling area	0.96	0.89	0.52	0.079
Frequency of any vacuuming in home	0.95	0.98	0.68	0.17
Frequency of any floor cleaning	0.99	0.65	0.48	0.094
Frequency of any windows open	0.84	0.88	0.45	0.11
Variables at sampling event				
Time since sampling area last vacuumed	1.0	0.85	0.41	0.085
Indoor relative humidity	0.97	0.49	0.37	0.020*
Outdoor relative humidity	0.93	0.65	0.37	0.023*
Indoor temperature	0.71	0.71	0.52	0.14
Outdoor temperature	1.0	0.89	0.61	0.067
Windows open	0.89	0.65	0.19	0.063
Carpet dust loading	0.89	0.93	0.41	0.15
Fixed home variables				
Age of home	0.82	0.88	0.97	0.38
Type of heat	1.0	0.86	0.35	0.092
Type of heat distribution	0.89	0.98	0.33	0.095
Home dampness index	0.72	0.99	0.58	0.027*
Number of bathrooms	1.0	0.84	0.46	0.030*
Number of houseplants in sampling area	0.94	0.82	0.37	0.091
Use of humidifier in home	0.87	0.74	0.42	0.091
Use of dehumidifier in home	1.0	0.64	0.37	0.10
Air conditioning in home	0.99	0.87	0.36	0.099
Basement in home	0.96	0.61	0.25	0.14
Pets in home	0.95	0.78	0.37	0.14
Carpeting height	0.94	0.70	0.18	0.078

$p < 0.05$ (*) indicates significant treatment effect of vacuuming group on fungal-spore concentration when co-variate was included in model.

(indoor and outdoor relative humidity at sampling, the home-dampness index and the number of bathrooms present in the home; Table 2). Eleven of the remaining 19 co-variables resulted in the vacuum-group treatment approaching significance ($0.05 < p \leq 0.1$). Including indoor relative humidity in the model resulted in a significant interaction between vacuuming group and sampling period, with essentially no difference among vacuuming groups during the June, September and November sampling periods, and a significantly lower mean spore concentration for the combined HEPA group compared to the control vacuum group during the April sampling period. The adjusted control-group geometric mean *Penicillium*/*Aspergillus* level in April exceeded the adjusted HEPA geometric mean by about 25-fold. Eighteen of 22 other interaction terms approached signifi-

cance ($0.05 < p \leq 0.1$) when single co-variables were included in the model explaining *Penicillium*/*Aspergillus* variation (Table 3). In all cases, the interaction was similar in direction and magnitude to the indoor relative humidity results, with a difference of approximately 10–30-fold in adjusted mean spore concentrations between the HEPA and control groups only during April.

4. Discussion

Combined over the entire study period, indoor airborne fungal-spore levels appeared unaffected by the type of vacuuming equipment used. Of the four spore categories, only the *Penicillium*/*Aspergillus* group showed a slight trend toward lower average spore concentrations in the groups using

Table 3. ANCOVA results – *p* values for tests of the effects of individual co-variables on significance of vacuuming-group $\times$ sampling-period interaction effect

Potential co-variables	<i>Ascomycetes</i>	<i>Basidiomycetes</i>	<i>Cladosporium</i> spp.	<i>Penicillium</i> / <i>Aspergillus</i> spp.
Cleaning-log variables				
Frequency of vacuuming in sampling area	0.47	0.55	0.34	0.073
Frequency of any vacuuming in home	0.50	0.57	0.40	0.089
Frequency of any floor cleaning	0.43	0.54	0.29	0.052
Frequency of any windows open	0.43	0.62	0.33	0.062
Variables at sampling event				
Time since sampling area last vacuumed	0.43	0.60	0.28	0.058
Indoor relative humidity	0.33	0.27	0.14	0.0046*
Outdoor relative humidity	0.50	0.70	0.13	0.11
Indoor temperature	0.26	0.72	0.30	0.053
Outdoor temperature	0.35	0.55	0.21	0.071
Windows open	0.50	0.91	0.22	0.075
Carpet dust loading	0.44	0.57	0.13	0.067
Fixed home variables				
Age of home	0.59	0.55	0.53	0.15
Type of heat	0.44	0.58	0.16	0.062
Type of heat distribution	0.46	0.56	0.14	0.059
Home dampness index	0.42	0.55	0.28	0.11
Number of bathrooms	0.48	0.55	0.36	0.12
Number of houseplants in sampling area	0.55	0.55	0.065	0.062
Use of humidifier in home	0.23	0.57	0.31	0.062
Use of dehumidifier in home	0.43	0.55	0.11	0.051
Air conditioning in home	0.46	0.55	0.14	0.066
Basement in home	0.49	0.56	0.20	0.053
Pets in home	0.56	0.56	0.14	0.082
Carpeting height	0.37	0.58	0.29	0.080

p < 0.05 (*) indicates significant treatment interaction effect on fungal-spore concentration when co-variate was included in model.

high-efficiency vacuum filtration compared to controls. Seasonality strongly influenced spore concentrations for all four spore categories. Mean indoor spore levels tended to decrease by at least 10-fold from mid-summer to late fall or winter during the study. These results are consistent with a strong influence of outdoor background spore levels on levels indoors.

When vacuuming group was considered within each sampling period, mean *Penicillium*/*Aspergillus* spp. concentrations tended to be lower for the HEPA vacuum groups than the control group during the late-winter period. There was a weak tendency for this pattern to hold for most of the other fungal-spore categories during April, although none approached statistical significance except the *Penicillium*/*Aspergillus* spp. result. Mean results for the control group during April

were about 2-fold to about 10-fold higher (depending on fungal-spore category) than the corresponding mean HEPA results from that sampling period. The largest mean difference was seen for the *Penicillium*/*Aspergillus* spp. results, where the mean control concentration exceeded the lower HEPA mean by about 20-fold. Results for the micro-filtration group did not differ significantly from the control results, even when stratified by sampling period. A third post-treatment sampling period was not part of the original study design and consequently only a limited number of homes were available for sampling during this period. The effect of HEPA vacuuming on *Penicillium*/*Aspergillus* spore levels during April might have appeared more statistically robust if more samples could have been collected during this period.

Airborne fungal-spore concentration indoors will reflect contributions from spores in outdoor air and spores from indoor sources. Indoor sources include dormant spores in the settled dust reservoir of carpeting, upholstered furniture and other surfaces and any active fungal growth that occurs indoors. Vacuuming is unlikely to have any effect on the contribution of outdoor background bioaerosols to indoor air levels, but could reduce the spore content of the settled dust reservoir. Vacuuming might also be effective in reducing spore emissions from fungal amplification associated with carpeting or other surfaces where high water activity supports fungal growth. However, this would probably depend on vacuuming frequency and the extent of fungal amplification. Controlling spore emissions from settled dust and surfaces indoors could have an appreciable indirect affect on indoor air levels by reducing the contribution of those sources under conditions where infiltration of outdoor fungal bioaerosols was limited. This effect would be most likely to be detected under conditions where ambient fungal bioaerosol levels are very low and relatively little infiltration of outdoor spores had occurred for several months, such as toward the end of winter in upstate New York. This is consistent with the results reported here of reduced airborne fungal-spore concentrations in homes using HEPA vacuums during the late winter sampling period, but not sampling periods in late summer or fall.

Several survey studies have shown that *Penicillium/Aspergillus* spores often make up a smaller proportion of the total outdoor airborne fungal-spore load, compared to their relative prevalence indoors, than other taxa (e.g., Li et al., 1995; Parat et al., 1997; Takahashi, 1997; Awad and Farag, 1999; Burr, 1999; Dharmage et al., 1999; Ren et al., 1999; Shelton et al., 2002). This suggests that indoor amplification in carpeting or other building materials may make a significant contribution to indoor levels for these genera. Indoor levels of these fungal taxa may be more susceptible to reduction by high-efficiency vacuuming since vacuum cleaning directly affects some indoor amplification sites. This is supported by the results reported here, where the effect of HEPA vacuuming on fungal air levels was most pronounced for the *Penicillium/Aspergillus* group.

Most variables considered in the study as potential covariates did not contribute significantly

to explaining airborne fungal-spore variability in the ANCOVA analyses. Indoor and outdoor relative humidity during sampling, whether any windows were open during sampling, and to a lesser degree, the average frequency of days with any windows open and the average frequency of vacuuming in the sampling area were the only variables that consistently contributed to explaining spore-count variation.

By including single covariates in the ANCOVA models, the vacuuming-group treatment either reached significance or approached significance for a majority of the variables tested for the *Penicillium/Aspergillus* spp. spore category. Those effects are independent of sampling period. Similarly, most of the vacuuming-group by sampling-period interaction terms approached or attained significance with the inclusion of a single co-variate in the model for this spore category. As with the crude ANOVA results, the effect of vacuuming group was more substantial in all these cases during April than during other sampling periods, with mean spore concentration in the combined HEPA group tending to be about an order-of-magnitude lower than in the control group. This also indicates that high-efficiency vacuuming can have some effectiveness in reducing exposure levels to fungi that have a significant contribution to their indoor concentrations from indoor spore production and where the effect of re-introduction of spores from external sources is minimized.

This study did not address the ability of high-efficiency vs. conventional vacuums to reduce exposure to airborne fungi or other particles during actual operation of the vacuum cleaner. Several previous studies conducted in the field and in controlled chamber environments have demonstrated that high-efficiency filtered vacuum cleaners do substantially reduce the amount of particulates released into the air from the vacuum's exhaust (Karla et al., 1990; Woodfolk et al., 1993; Hegarty et al., 1995; Green et al., 1999; Lioy et al., 1999; Vaughan et al., 1999). High-efficiency two- or three-ply vacuum bags effectively reduced particles emissions in some of these studies, as did vacuums with HEPA or passive-electrostatic exhaust filters. In contrast, Durrell et al. (2001, 2002) report in recent conference abstracts that personal inhalation exposure to dust-borne antigens (assessed via nasal sampling; Graham et al., 2000; Mitakakis et al., 2000) might be increased for

someone actually operating a vacuum cleaner, even when the vacuum has HEPA exhaust filtration. The action of the beater-bar dispersing particles out from beneath the vacuum cleaner was suggested as a possible mechanism for this observed effect. However, simply walking for 1 minute on a carpet pre-loaded with *Penicillium chrysogenum* spores raised the airborne spore level about 10-fold over undisturbed conditions in a chamber study (Buttner and Stetzenbach, 1993). So, another possibility is that disturbance of the settled dust reservoir caused by the motion of the vacuum and the operator over the carpet surface is being detected by this sampling method. The effectiveness of HEPA-filtered vacuums to control allergen exposures during vacuuming needs further investigation.

In conclusion, fungal-spore concentrations in air were not dramatically affected by the routine use of HEPA or micro-filtration vacuums in homes, compared to routine use of conventionally filtered vacuum cleaners. However, the results suggest that, under conditions where most of the fungal spores present indoors derive from spore production in carpeting or in the settled-dust reservoir itself, high-efficiency vacuuming could cause some reduction in long-term average spore concentrations in indoor air by effectively reducing spore loading in the settled-dust reservoir while having little replacement of spores from external sources such as outdoor air. This suggests that using a vacuum with high-efficiency filtered exhaust could have some (perhaps modest) effectiveness in controlling airborne fungal-spore exposure in homes where measures are routinely taken to limit infiltration of unfiltered outdoor air.

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