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Monitoring Airborne Fungal Spores in an Experimental Indoor Environment To Evaluate Sampling Methods and the Effects of Human Activity on Air Sampling

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Aerobiological monitoring was conducted in an experimental room to aid in the development of standardized sampling protocols for airborne microorganisms in the indoor environment. The objectives of this research were to evaluate the relative efficiencies of selected sampling methods for the retrieval of airborne fungal spores and to determine the effect of human activity on air sampling. Dry aerosols containing known concentrations of *Penicillium chrysogenum* spores were generated, and air samples were taken by using Andersen six-stage, Surface Air System, Burkard, and depositional samplers. The Andersen and Burkard samplers retrieved the highest numbers of spores compared with the measurement standard, an aerodynamic particle sizer located inside the room. Data from paired samplers demonstrated that the Andersen sampler had the highest levels of sensitivity and repeatability. With a carpet as the source of *P. chrysogenum* spores, the effects of human activity (walking or vacuuming near the sampling site) on air sampling were also examined. Air samples were taken under undisturbed conditions and after human activity in the room. Human activity resulted in retrieval of significantly higher concentrations of airborne spores. Surface sampling of the carpet revealed moderate to heavy contamination despite relatively low airborne counts. Therefore, in certain situations, air sampling without concomitant surface sampling may not adequately reflect the level of microbial contamination in indoor environments.

Human exposure to airborne microorganisms may result in a variety of adverse health effects, including infectious diseases (4, 21), allergic and irritant responses (3, 11, 13), respiratory problems (9, 11, 15, 21, 30), and hypersensitivity reactions (12, 20, 29). Airborne microorganisms have been identified and enumerated by using a variety of aerobiological sampling methods (17, 19, 24, 28, 29), yet the impact of airborne microorganisms on indoor air quality and human health remains poorly understood. This is due in part to difficulties with aerobiological sampling methodology and the interpretation of experimental data. Although several methods for monitoring airborne microorganisms are commercially available (10, 26, 27), there is a lack of information concerning the comparability of these methods. The American Conference of Governmental Industrial Hygienists has outlined several sampling methodologies for indoor monitoring but has not reported the comparability of data derived from the various methods (1). Confusion and criticism of sampling methods arise during interpretation of field data as accuracy determinations are rarely made during aerobiological sampling surveys (6) and the sampling method used may not be appropriate for the survey being conducted (8). Difficulties also arise with the interpretation of data because of numerous variables present in indoor environments, such as the activity of building occupants near the air sampling site. Although foot traffic has been shown to affect airborne concentrations of microorganisms during sampling (14), the extent of human activity on sampling has not been fully investigated.

As a step toward the development of standardized sampling protocols and to provide information concerning the impact of airborne microorganisms on indoor air quality, research was conducted in an experimental room designed as a microcosm of a residential indoor environment. The objectives of this work were to determine the relative efficiencies of selected aerobiological sampling methods and the effect of human activity on retrieval of airborne microorganisms. In these experiments, fungal spores of *Penicillium chrysogenum* (spherical spores with a known size range) were aerosolized, their concentrations were monitored by using a laser-based aerodynamic particle sizer (APS), and the results were compared with data from total count and culture-based sampling methods.

MATERIALS AND METHODS

Experimental room. An experimental room (4 m long by 4 m wide by 2.2 m high) was designed to resemble a residential indoor environment. The interior of the room was fitted with a hardwood floor, and the sheetrocked walls and ceiling were coated with interior latex paint. The room was equipped with a heating, ventilation, and air conditioning system that was sized to simulate a residential system and had rectangular ductwork (13 by 20 cm). The room was designed as a closed system with two registers (10 by 20 cm) (Fig. 1, S1 and S2), which were located at a height of 1.8 m off the floor and were 1.8 m apart; these registers supplied HEPA-filtered (99.97% efficiency) air, and there was one return vent (25 by 30 cm) (R), Fig. 1, which was located 30 cm off the floor on the opposite wall. The room was operated at a temperature of 24 ± 0.3°C with an air flow rate of 4.2 m^3/min, which resulted in approximately five room air volume exchanges per hour. The duct velocity was approximately 168 m/min. The room and supply ducts were maintained at a positive static pressure (0.5 mm of water) to minimize contamination from

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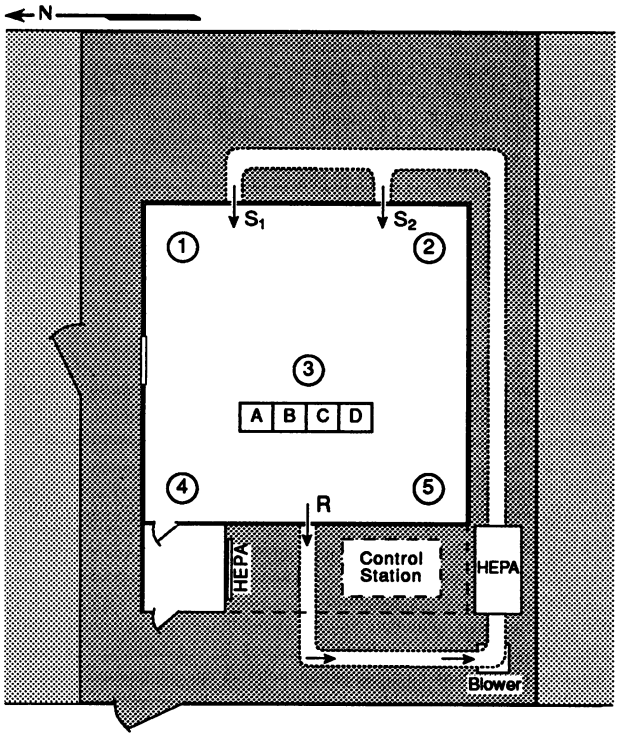

FIG. 1. Diagram of the experimental room. The arrows indicate the direction of air flow. HEPA-filtered air is delivered into the room via two supply registers (S_1 and S_2) and exits through the return register (R). Sampling stands (locations 1 through 5) support temperature and humidity probes. See the text for an explanation of the positions of the APS, Andersen, and Burkard samplers (locations A through D).

outside the room. An anteroom equipped with a HEPA-filtered air shower was attached to the room entrance to reduce mixing of air resulting from entering and exiting during experiments. The temperature was monitored by 20 type T thermocouples (Thermo Electric Co., Saddle Brook, N.J.), and the relative humidity was monitored by five relative humidity probes (Hy-Cal Engineering, El Monte, Calif.) located within the room (Fig. 1).

Target organism and culture media. Spores of the fungus *P. chrysogenum* were obtained from Harriet Burge, University of Michigan Medical Center. This organism is a common fungal isolate in indoor air samples and is not known to be a human pathogen or a producer of mycotoxins that affect humans. Spores were stored dry at 4°C until they were needed. *P. chrysogenum* was cultured on malt extract agar (MEA) (pH 4.5) (Difco Laboratories, Detroit, Mich.) and was incubated at 23°C for 72 h. The bacteria present in the experimental room were cultured by using tryptic soy agar (Difco) (pH 7.0) supplemented with 100 μg of cycloheximide per ml and incubated at 28°C for 5 days.

Aerobiological sampler evaluation. *P. chrysogenum* spores were introduced into the room via the air supply duct at supply register S_1 (Fig. 1) by using a Pitt 3 dry aerosol generator (25). For each trial, a dry aerosol of spores was generated inside a sealed Plexiglas column by acoustic vibration. Filtered, dry CO_2 gas conveyed the spores from the Pitt 3 generator to the supply duct at a rate of 10 liters/min for 10 min or until the spore concentration in the room reached 10^3 spores per m^3, as measured by an APS (TSI, Inc., St. Paul, Minn.) placed inside the room for real-time determination of fungal spore concentrations (18, 25). The APS is capable of measuring particles in the size range from 0.5 to 30 μm and operates at an air flow rate of 5 liters/min. The spore concentration was determined by counting the particles in the *Penicillium* spore size range (1.8 to 3.5 μm). Background levels of particles in this size range were measured by using the APS before spores were released.

Four types of aerobiological samplers, which were activated remotely from outside the room, were evaluated for the retrieval of airborne fungi. Two Andersen six-stage viable impactor samplers (Andersen Instruments, Inc., Atlanta, Ga.) were operated for 5 min at a flow rate of 28 liters/min by using vacuum pumps (Millipore Corp., Bedford, Mass.) located outside the room; a total of 0.140 m^3 of air was sampled. Two Surface Air System (SAS) viable impactor samplers (manufactured by PBI International, Milan, Italy, and distributed by Spiral Biotech, Inc., Bethesda, Md.) were fitted with 487-hole faceplates and large-diameter (84-mm) RODAC plates (PBI International) and were operated for 1 min. The SAS batteries were fully charged before each trial, and the flow rates of the two samplers were calibrated in our laboratory at 153 and 159 liters/min; the amounts of air sampled were 0.153 and 0.159 m^3, respectively. Fifteen pairs of 100-mm agar-filled petri dishes were placed in remotely activated depositional samplers (designed and manufactured by Owens-Corning Fiberglas, Granville, Ohio) and were exposed for 15 min by using pneumatic switches which slid trays containing the petri dishes from underneath plastic covers to expose the agar surfaces. A single Burkard spore trap (Burkard Manufacturing Co., LtD., Rickmansworth, Hertfordshire, England) was fitted with a 24-h sampling head that held an adhesive-coated glass slide onto which fungal spores were impacted. The Burkard sampler was operated for 30 min at a flow rate of 10 liters/min (total air volume, 0.30 m^3).

A total of 12 trials were conducted to evaluate the results of the aerobiological sampling methods compared with the APS data. The depositional samplers were placed at positions that were 0.3, 1, and 1.5 m from the floor on each of five sampling stands that were located in the room; a total of 30 plates were used for each trial (Fig. 1, locations 1 through 5). The APS was placed on a sampling cart near the center of the room at a height of ca. 1 m from the floor (Fig. 1, location A). The Andersen, SAS, and Burkard samplers were positioned in line with the APS (Fig. 1, locations B through D); the Andersen and SAS sampling ports were ca. 1 m from the floor, and the floor-mounted Burkard sampling port was ca. 0.3 m from the floor. The location of each sampler type relative to the APS was changed between trials such that all six possible arrangements were tested twice (Table 1).

After the room was loaded with spores, the air-handling system was turned off, and 5 min was allotted for the room air to equilibrate. Air samples were taken with the pairs of each viable sampler type operated in sequence. On the basis of predicted settling rates of the spores (as determined by terminal velocity approximations) and observations with the APS, the total length of the sampling period was such that the majority of the aerosolized spores remained airborne during the entire sampling period. The order of operation of the Andersen, SAS, and depositional samplers was changed between trials, and each order was tested twice (Table 1). The Burkard sampler exhausts air near the floor and reentrains particles which interfere with the APS measurements. Therefore, the Burkard sampler was operated last in

TABLE 1. Air sampler locations and order of operation for aerobiological sampler evaluation

Trial no.	Andersen sampler		Burkard sampler		SAS sampler		Depositional sampler: order in sequence[c]
	Location[a]	Order in sequence[b]	Location[a]	Order in sequence[b]	Location[a]	Order in sequence[b]	
1	B	3	C	4	D	1	2
2	B	1	C	4	D	3	2
3	B	1	D	4	C	2	2
4	B	2	D	4	C	1	3
5	C	2	B	4	D	1	3
6	C	1	B	4	D	2	3
7	D	3	B	4	C	2	1
8	D	2	B	4	C	3	1
9	C	1	D	4	B	3	2
10	C	3	D	4	B	1	2
11	D	2	C	4	B	3	1
12	D	3	C	4	B	2	1

[a] See Fig. 1.
[b] The order in which the samplers were operated was varied from trial to trial (see text).
[c] The depositional sampler locations were fixed (see Fig. 1 and text).

every trial. The APS collected continuous 5-min samples at an air flow rate of 5 liters/min throughout the experiment.

Air sample analysis. The MEA plates from the Andersen, SAS, and depositional samplers were incubated as described above, and fungal counts were determined as the number of colony-forming units per plate. The number of viable *P. chrysogenum* spores per m^3 of air was determined for each Andersen and SAS sampler by using the appropriate air flow rates and sampling times. The mean number of colony-forming units per cubic meter for each pair of samplers was then calculated. The data from paired depositional samplers were expressed both as mean number of colony-forming units per plate and as mean number of colony-forming units per square meter of collection surface area. Each Burkard slide was stained by using the standard formulation developed by Harriet Burge (5a), except that crystal violet was substituted for phenosafranin. The total number of *Penicillium* spores was determined microscopically. A total of 8 to 10 microscopic sweeps was made for each slide, and the average count per sweep was used to estimate the number of total spores per cubic meter of air. The particle counts from the APS in the *P. chrysogenum* spore size range were determined and converted to total number of particles per cubic meter of air.

Fungal spore viability. The percentage of viable *P. chrysogenum* spores released into the room was estimated for each spore release trial. A sample of spores was removed from the Pitt 3 generator after aerosol generation and placed in filtered Isoton II solution (Coulter Electronics, Hialeah, Fla.). After the sample was sonicated for 10 min with a model 1200 sonicator (Branson Ultrasonics Corp., Danbury, Conn.), the total number of spores per milliliter was determined by using a Coulter Counter ZM electronic particle counter (Coulter Electronics, Inc.) fitted with a 30-μm orifice and calibrated with 2.02-μm-diameter latex beads. The number of viable spores per milliliter was determined by serially diluting the preparation and spread plating the spore suspension onto triplicate MEA plates. The MEA plates were incubated as described above, and the mean number of colony-forming units for triplicate plates was determined. The percentage of viable spores released in each trial was then estimated.

Air sampling with human activity. Trials were conducted to determine the effect of human activity on airborne spore retrieval by using wall-to-wall carpet as the source of fungal spore contamination. A nylon synthetic pile carpet was installed in the room, and the surfaces of the carpet, walls, and ceiling were tested to determine background levels of nontarget fungi and bacteria by using RODAC contact plates filled with MEA and tryptic soy agar (pH 7.0) containing 100 μg of cycloheximide per ml, respectively. *P. chrysogenum* spores were introduced into the room via the air supply duct as described above and settled onto the carpet. The average airborne spore concentration during spore loading was determined by the APS to be 10^4 spores per m^3.

The aerobiological samplers were placed in the room by a scientist wearing a respirator and protective clothing. Following a minimum of 3 h of settling time, air samples were taken in the undisturbed room with each type of sampler operated individually, in the following sequence: SAS samplers, Andersen samplers, depositional samplers, and Burkard sampler. Immediately following aerobiological sampling in the undisturbed room, the Andersen samplers in the room were replaced with unexposed Andersen samplers, and SAS and depositional samplers were reloaded with unexposed agar plates. An additional type of viable impactor sampler, the RCS Plus sampler (Biotest Diagnostics Corp., Denville, N.J.) containing MEA strips, was added to the array of samplers. This sampler was not equipped to operate under the experimental conditions of the sampler efficiency study described above or under the undisturbed conditions in these trials. Because of an automatic shutoff, the RCS Plus sampler required activation within 4 min and therefore could not be operated remotely. The RCS Plus sampler was operated for ca. 3 min and sampled 0.14 m^3 of air. After the samplers were replaced, human activity was initiated. The room air was disturbed by walking of the scientist in the room along a preselected pattern for approximately 1 min. Two RCS Plus samplers were activated manually immediately before the scientist left the room, and the other samplers were then operated remotely in the same sequence as described above for the undisturbed conditions. Five trials were performed, in which air samples were taken under both undisturbed and disturbed conditions.

This series of experiments was repeated after the room was loaded with a higher concentration of spores (10^5 spores per m^3 [average concentration]). In five trials with the more heavily contaminated carpet as the spore source, both undisturbed room air and air disturbed by walking were sam-

pled, as described above. Five trials were also performed at this level of contamination in which vacuuming (upright household vacuum cleaner equipped with conventional paper vacuum bags) for 2 min replaced walking as the human activity disturbance. MEA-filled RODAC contact plates were used to quantify the viable *P. chrysogenum* spores on the walls, ceiling, and carpet throughout the human activity trials.

Statistical analysis. Data obtained as the number of colony-forming units per plate from aerobiological sampling were converted to the number of colony-forming units per cubic meter of air for the Andersen, SAS, and Burkard samplers or the number of colony-forming units per square meter of surface area for the depositional samplers. Data from the Andersen six-stage and SAS samplers were recorded both as uncorrected counts and as positive-hole-corrected (2) and coincidence-corrected counts (16), respectively. The data were $\log_{10}$ transformed, and all subsequent analyses were performed by using the transformed data. Analysis of variance (ANOVA) calculations were performed by using the counts from forced-air-flow samplers to determine the effects of the following factors: (i) the location of the sampler relative to the APS, (ii) the order of operation of a sampler type in the sampling sequence, (iii) the differences between paired samplers of the same type operated simultaneously (excluding the Burkard sampler), and (iv) the differences between sampler types, where the measurement units coincided (Andersen sampler versus SAS sampler and Burkard sampler versus APS). Data from depositional sampling were analyzed by ANOVA to determine the effects of sampler height and location and the differences between sample pairs.

Sampling methods were compared by performing a correlation analysis and by determining levels of repeatability and sensitivity. Estimates of correlation values were obtained by pairing the sample mean for the two observations for one sampler type with the corresponding mean for another sampling type for each trial. The data were ranked, and Spearman's rank correlation coefficients were calculated. The advantages of using rank correlations rather than product moment correlations are that the rank correlations do not rely on the assumption of bivariate normal data and measure the extent of a monotonic relationship rather than the extent of a linear relationship (25a). The level of repeatability (the similarity of measurements made by two samplers of the same type operated simultaneously) for each sampling method except the Burkard method was obtained in the following two ways: (i) by calculating the mean of the differences (absolute value) between two samplers of the same type operated simultaneously and (ii) by estimating the pooled within-duplicate-pair variance, which was obtained as the error mean square in an ANOVA of the data for each type of sampler. The sensitivity estimates are the F ratios of the between-location mean square to the error mean square for each type of sampler, which provide a way to compare how well different sets of measurements from the same sampling method distinguish between different sampling conditions (5).

For air sampling trials with human activity, the paired-sample *t* test was used to compare the data for the undisturbed conditions with the data for the disturbed conditions for each sampler type. A one-way ANOVA was used to compare the Andersen, SAS, and RCS Plus sampler data obtained after disturbance by human activity. Depositional sampler data from the three different sampling heights were also compared by using the one-way ANOVA.

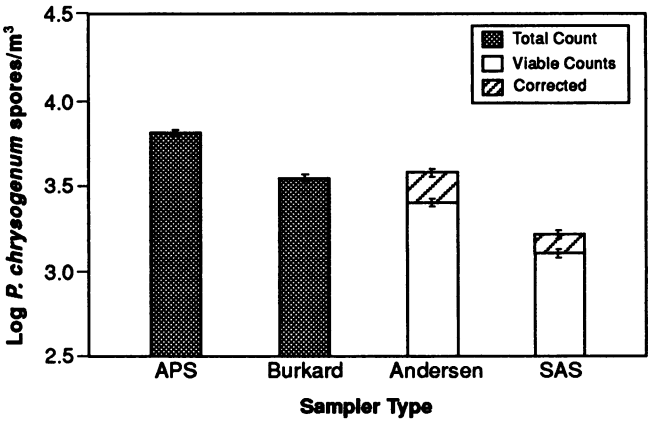

FIG. 2. Comparison of aerobiological sampling methods. For each sampler type (*x* axis) the bar height represents the mean (n = 12) level of retrieval of *P. chrysogenum* spores ± 1 standard error (data are expressed as the log of number of spores per cubic meter of air). APS and Burkard sampler data are total spore counts. Andersen and SAS sampler data are viable spore counts expressed as both uncorrected and corrected values.

RESULTS

Aerobiological sampler evaluation. Electronic particle counts of spores obtained by using the Coulter Counter and preliminary release trials monitored by the APS indicated that ≥95% of the *P. chrysogenum* spores were 1.8 to 3.5 μm in diameter (data not shown). Therefore, only particles in this size range were recorded with the APS as *P. chrysogenum* spores during the spore release trials. The average spore concentration per release in the 12 trials was 6.4×10^3 spores per m^3 of air, and the range was $4.5 \times 10^3 \pm 295$ spores per m^3 (mean ± 1 standard deviation) to $7.8 \times 10^3 \pm 390$ spores per m^3. The background concentration of particles in the spore size class prior to spore release ranged from below the lower detection limit (71 particles per m^3) to 143 particles per m^3. During release of spores, the air flow rate through the room was 4.2 m^3/min, the room temperature was 23.6 to 24.4°C, the relative humidity was 50 to 56%, and the room pressure was 0.5 mm of water. After air sampling was completed (the air-handling system was off), the temperature in the room was 24.9 to 26.3°C and the relative humidity ranged from 48 to 53%.

The results of the experiments to determine *P. chrysogenum* spore retrieval by the forced air flow sampling methods and the APS data for comparison are shown in Fig. 2. The Andersen sampler retrieved a significantly higher number of viable *P. chrysogenum* spores than the SAS sampler, both before and after coincidence correction ($P < 0.0001$). The APS estimates of total spore concentration in the room were significantly higher than the total concentration retrieved by the Burkard sampler ($P < 0.0001$). The level of spore retrieval in the 12 trials in which the depositional samplers were used was 6.8 ± 0.5 CFU per plate (mean ± 1 standard error), or 1,117 ± 85 CFU/m^2 of collection surface area. The level of spore viability was 74.8 ± 10.5% in these trials.

Levels of repeatability and sensitivity were estimated for the Andersen, SAS, and depositional sampling methods (Table 2). For repeatability, the lower the values for either the mean difference between pairs or the variance, the greater the level of repeatability between samplers of that type. F values indicate the level of sensitivity of a sampling

TABLE 2. Repeatability and sensitivity measurements for the Andersen, SAS, and depositional samplers

Sampler	Repeatability		Sensitivity: F-value
	Mean difference between pairs	Variance	
Andersen			
Uncorrected data	0.0200	0.0003	37.41
Corrected data[a]	0.0281	0.0006	39.08
SAS			
Uncorrected data	0.1061	0.0071	2.51
Corrected data[b]	0.1442	0.0134	2.18
Depositional	0.2010	0.0379	1.83

[a] Andersen positive-hole correction.
[b] SAS coincidence correction.

method to differences between trials. The higher the F value, the greater the level of sensitivity of the method (25a). The Andersen sampler exhibited both the highest level of repeatability and the greatest level of sensitivity in these experiments, followed by the SAS sampler. The depositional samplers exhibited the lowest levels of repeatability and sensitivity. Correction of Andersen and SAS sampler data emphasized the differences between sampler pairs which resulted in a slight decrease in the level of repeatability for both samplers. Corrected values resulted in an increase in the level of sensitivity of the Andersen sampler, but a decrease in the level of SAS sampler sensitivity.

The results of the correlation analysis between sampler types are shown in Table 3. After positive-hole correction, the Andersen sampler was the only sampler type that showed a significant correlation with the APS.

There were no significant differences among the results obtained from different sampling locations for the Andersen ($P = 0.822$ uncorrected; $P = 0.840$ corrected), SAS ($P = 0.617$ uncorrected; $P = 0.602$ corrected), Burkard ($P = 0.942$), and depositional samplers ($P = 0.267$). For depositional sampling, there were also no differences among data obtained from the 0.3-, 1-, and 1.5-m sampling heights ($P = 0.405$) (data not shown). However, there were significant differences in the results obtained depending on the order in

TABLE 3. Correlation between aerobiological sampler data and APS

Sampler	Spearman's rank correlation coefficient with APS data
Andersen	
Uncorrected data	0.584
Corrected data[a]	0.656[b]
SAS	
Uncorrected data	0.436
Corrected data[c]	0.436
Burkard	0.425
Depositional	0.510

[a] Andersen positive-hole correction.
[b] Correlation is significantly different from zero at the 5% level of significance.
[c] SAS coincidence correction.

TABLE 4. Surface sample results prior to human activity trials for each of the three experimental conditions

Room contamination release concn (spores/m^3)	Activity	Location	No. of samples	CFU/plate (mean)	CFU/m^2 (mean)
10^4	Walking	Carpet	24	30.7	12,019
		Walls	24	0.2	65
10^5	Walking	Carpet	15	>300	>117,576
		Walls	13	1.8	724
10^5	Vacuuming	Carpet	18	196	76,729
		Walls	18	4.7	1,851

which the sampler types were operated. When the Andersen sampler was operated third in the sampling sequence, the counts were significantly lower ($P = 0.020$ uncorrected; $P = 0.018$ corrected) than the counts for this sampler when it was operated first or second. The order of operation in the sampling sequence did not result in a significant difference in the SAS sampler data ($P = 0.061$ uncorrected; $P = 0.067$ corrected). There were no significant differences in the results obtained from paired samplers of the same type when the Andersen samplers ($P = 0.710$ uncorrected; $P = 0.662$ corrected) and the depositional samplers ($P = 0.147$) were used. However, one of the SAS samplers retrieved consistently higher numbers of spores than the other SAS sampler; this difference was significant following coincidence correction of the data ($P = 0.057$ uncorrected; $P = 0.048$ corrected).

The average background bacterial concentration in the room was 41.6 CFU/m^3 for the 12 release trials. Low numbers of spores were detected in the greenhouse surrounding the experimental room; the average *Penicillium* count was 6.7 CFU/m^3, and the average total fungus count was 305.7 CFU/m^3.

Air sampling with human activity. Aerobiological sampling was conducted in the experimental room before and after disturbance by human activity, using carpet as the source of *P. chrysogenum* spore contamination. The APS measured a mean concentration of 10^4 spores per m^3 in the experimental room during the initial spore release. After the spores settled, the average surface count from the carpet was 30.7 CFU per plate, or ca. 1×10^4 CFU/m^2, prior to air sampling (Table 4). Trials were then conducted in which air samples were taken under both undisturbed conditions and after a person walked in the room (Fig. 3A). Low numbers of airborne spores were recorded at this level of carpet contamination, and walking did not result in a significant increase in spore concentration, as measured by the Andersen sampler ($P = 0.14$). However, the Burkard ($P = 0.015$) and SAS ($P = 0.023$) samplers detected significant increases in the numbers of airborne spores after walking. The depositional sampler data exhibited no significant increase in spore counts after walking ($P = 0.20$) (Table 5).

Trials were then conducted with an increased loading of *P. chrysogenum* spores (10^5 spores per m^3), which resulted in carpet surface counts of ≥300 CFU per plate, or ca. 1×10^5 CFU/m^2 (Table 4). With this increased spore load in the carpet, disturbance by walking resulted in the retrieval of significantly higher ($P < 0.05$) concentrations of airborne spores than were obtained with the undisturbed conditions for all sampler types (Fig. 3B and Table 5). For trials conducted with vacuuming as the disturbance, surface samples from the carpet taken prior to air sampling averaged 196

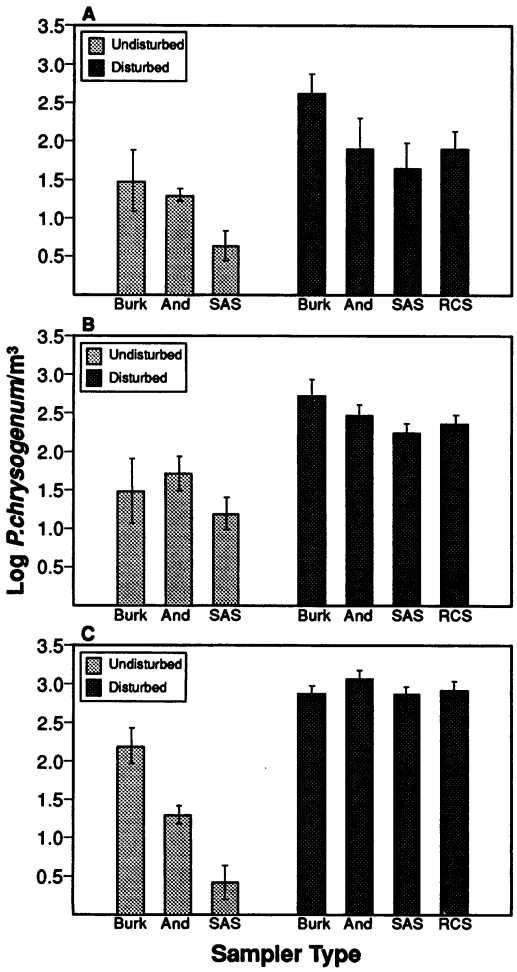

FIG. 3. Effect of human activity on airborne spore concentration compared with undisturbed room air. (A) Walking on a moderately contaminated carpet (spore release concentration, 10^4 spores per m^3). (B) Walking on a heavily contaminated carpet (spore release concentration, 10^5 spores per m^3). (C) Vacuuming a heavily contaminated carpet. The bar heights are the means ± 1 standard error for five trials. Burk, Burkard sampler; And, Andersen sampler; RCS, RCS Plus sampler.

CFU per plate, or ca. 8×10^4 CFU/m^2 (Table 4). Vacuuming the carpet resulted in a significant increase in the airborne spore concentration compared with the undisturbed conditions ($P < 0.05$) (Fig. 3C and Table 5) and had a greater effect on airborne spore concentration than walking at this level of surface contamination.

TABLE 5. Depositional sampler data from human activity experiments

Room contamination release concn (spores/m^3)	Activity	Undisturbed		Disturbed	
		Mean CFU/plate	Mean CFU/m^2 (±1 SE)	Mean CFU/plate	Mean CFU/m^2 (±1 SE)
10^4	Walking	0.07	10.96 ± 3.88	0.48	78.9 ± 44.7
10^5	Walking	0.13	21.92 ± 8.31	0.67	110.7 ± 33.1
10^5	Vacuuming	0.04	6.58 ± 3.20	2.91	478.0 ± 141.0

The data obtained by using the RCS Plus sampler following disturbance in the room were comparable to the Andersen and SAS sampler data in these trials, as no significant difference was observed among the data from these sampling methods for any of the three experimental conditions (Fig. 3). Depositional samplers demonstrated the same trend as the forced-air-flow samplers in these trials. In addition, no significant differences were observed among the depositional samplers placed at heights of 0.3, 1.0, and 1.3 m for all trials under both undisturbed and disturbed conditions (data not shown). No colonies of *P. chrysogenum* were obtained from surface samples taken from the ceiling, and wall counts were low compared with carpet counts under all of the conditions tested.

DISCUSSION

The Burkard and Andersen samplers were the most accurate samplers tested in these trials for retrieval of spores in the size range from 1.8 to 3.5 μm at an airborne concentration of 10^3 spores per m^3. However, because an estimated 25% of the spores were not viable and the Andersen and SAS samplers used in this project measure only viable spores, the fungal concentrations in the room as measured by the Andersen and SAS samplers were underestimated. When data from paired samplers of the same type were compared, the Andersen sampler had the highest levels of sensitivity and repeatability, followed by the SAS sampler. Depositional samplers had the lowest levels of repeatability and sensitivity. Under the controlled conditions in the experimental room, the level of repeatability was low overall for aerobiological samplers. The mean differences between data from paired samplers of the same type for the concentrations measured in these experiments (Table 2) show that the paired Andersen sampler values differed by approximately 7% on average after positive-hole correction. The paired values from the SAS and depositional samplers differed by averages of 25 and 37%, respectively. Lembke et al. (17) evaluated the precision of the Andersen six-stage sampler for retrieval of airborne bacteria in solid-waste handling facilities. Although the data in that study showed that there was a linear relationship between paired-sampler data for concentrations of 10^3 to 10^5 bacterial CFU per m^3, there was a high degree of variability between paired samples. In addition, preliminary information from a recent study in our laboratory (unpublished data) indicated low levels of repeatability and sensitivity when fungal concentrations in homes were measured. Variables such as size class of microorganisms sampled and operation of the air-handling system also affected these parameters. The lower airborne fungal counts recorded when the Andersen samplers were operated last in the sequence may have been due to a combination of the decay of the bioaerosol over time and the sensitivity of the Andersen samplers for detecting changing conditions. The greater retrieval of fungi with one of the SAS samplers is unexplained. Therefore, a single air sample at a single location at a discrete point in time may have limited value when the concentration of viable airborne microorganisms is assessed with currently available samplers because of the lack of repeatability and sensitivity.

The Burkard sampler could not be evaluated for repeatability and sensitivity because only a single sampler was operated. However, the Burkard spore trap sampler does provide a way to sample air for long periods of time to determine temporal effects on airborne fungal spore concentrations. The Burkard spore trap sampler also was the only

aerobiological sampler tested which permitted determination of total numbers of airborne *P. chrysogenum* spores, eliminating the reliance on spore viability for detection. Determination of total spore numbers is desirable because allergic and hypersensitivity reactions may be caused by the presence of fungal antigens and may not be linked to the viability of the fungus (1). However, the spore concentrations determined by using the Burkard sampler were lower than the APS estimate by an average of 45%. This lack of accuracy may have been due in part to a low efficiency of collection of particles in the *P. chrysogenum* spore size range. Other potential problems with Burkard samples are errors associated with enumerating spores microscopically and in distinguishing them from debris, problems which can become magnified in field situations with mixed populations of spores. The APS was used as a standard measurement method in this study to determine the spore concentrations during the spore releases and to give a real-time estimate of the true concentration of airborne spores in the room. However, HEPA-filtered input air was required to minimize background particle levels, and it is impractical to use an APS in most field situations for aerobiological sampling because of the presence of numerous airborne particles from a variety of sources in nonfiltered environments. Furthermore, because increased variance is observed with low particle counts, the use of an APS as a measurement standard for microorganisms when the airborne concentration of microorganisms is less than 10^3 particles per m^3 is impractical at this time.

The Andersen positive-hole correction and the SAS coincidence correction are estimates that are based on the probability of multiple impacts through the same sampling hole which result in enumeration of a single colony. The corrected data were more similar to the APS measurements in these trials, which resulted in a significant correlation between the Andersen sampler data and the APS data (Table 3). However, correction of the data magnified the differences between paired samplers, which reduced the level of repeatability slightly and had a mixed effect on the level of sensitivity for the Andersen and SAS samplers (Table 2). For fungal spores in the size range and concentrations measured in this experiment, use of the corrections was advantageous in terms of concentration estimates, and there was a slightly negative effect on the level of repeatability. However, the magnitude of these corrections increases exponentially with increasing colony counts. Therefore, measurements of airborne fungal spores at higher concentrations in the experimental room will be necessary to determine the concentration range at which the correction may be usefully employed.

In these experiments, a large 487-hole SAS faceplate and 84-mm-diameter RODAC plates were used in place of the standard 219-hole faceplate and standard RODAC plate. This raised the upper quantitation limit, allowing a longer sampling time of 1 min. To our knowledge, the SAS sampler has not been evaluated previously for the retrieval of airborne fungi by using the large faceplate.

Depositional sampling relies on the settling of airborne microorganisms onto agar-filled petri dishes or sticky film. This is a low-cost alternative to forced-air-flow sampling methods, but microorganisms deposited on the sampling surface may not be representative of all viable cells and fungal spores in the air (7, 22, 23). Sayer et al. (22, 23) compared the six-stage sampler with depositional settling for the retrieval of spores and reported that gravity settling plates do not compare favorably for quantitative studies of airborne fungi or bacteria in the hospital environment. These experiments in the room were conducted in a nearly optimum environment for depositional sampling. The bioaerosol was approximately uniform in size and high in concentration, the air-handling system was turned off, and the samplers were activated remotely without human activity. Yet, the levels of repeatability and sensitivity for the depositional sampler were the lowest values for all of the paired aerobiological samplers tested. In addition, the numbers of colonies per plate obtained from depositional sampling were low considering the relatively high bioaerosol concentrations generated in these experiments. For example, a mean of 6.8 CFU per plate was retrieved on depositional samplers in the sampler evaluation trials when the mean airborne spore concentration as measured by the APS was 6.4×10^3 spores per m^3. Similarly, in human activity trials after vacuuming, the mean count on depositional samplers was only 2.9 CFU per plate when forced-air-flow sampler data estimated ca. 10^3 spores per m^3. Therefore, the uncertainties involved with depositional sampling preclude reliable quantitative assessment of airborne microorganisms in noncontrolled environments.

Human activity in and around the sampling site is common during indoor air investigations, and questions have been raised concerning the significance of these disturbances when concentrations of airborne microorganisms are measured. Previously, foot traffic was shown to increase the airborne concentrations of bacteria (14). Our results with human activity on carpet contaminated with fungal spores support the results of this previous work. Airborne counts of the magnitude reported in the experimental room are generally considered within an acceptable range (1, 18), yet both of the indoor environments tested contained moderate to heavy levels of contamination with fungi, as determined by surface samples. Only the trials with vacuuming, which created a considerable disturbance, produced airborne concentrations considered to be high. Therefore, when the source of indoor microorganisms is a carpet, the collection of air samples without concomitant surface sampling may not adequately reflect microbial contamination in indoor environments.

In this research project conducted with an experimental room, several sampling methods for indoor air monitoring of fungi were compared. For *P. chrysogenum* spores at an airborne concentration of 10^3 spores per m^3, the Andersen six-stage and Burkard samplers were the most accurate samplers when the data were compared with the APS measurements. Of the paired samplers tested, the Andersen samplers demonstrated the highest levels of repeatability and sensitivity. Coincidence correction of Andersen and SAS sampler data improved concentration estimates, but resulted in a slight reduction in the level of repeatability. Human activity resulted in significant increases in airborne concentrations of spores and also demonstrated a potential limitation of air sampling for adequately assessing microbial contamination in indoor environments. In order to establish an indoor sampling protocol for the retrieval of microorganisms, further research needs to be conducted to evaluate aerobiological sampler efficiency with additional aerosol concentrations and particle size ranges. In addition, surface sampling methodologies should be tested to determine their effectiveness for detecting microbial contamination in the indoor environment.

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REFERENCES

1. **American Conference of Governmental Industrial Hygienists.** 1989. Guidelines for the assessment of bioaerosols in the indoor environment. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio.
2. **Andersen, A. A.** 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. J. Bacteriol. **76:**471–484.
3. **Bernstein, I. L., and R. Safferman.** 1973. Clinical sensitivity to green algae demonstrated by nasal challenge and *in vitro* tests of immediate hypersensitivity. J. Allergy Clin. Immunol. **51:**22–28.
4. **Bollin, G. E., J. F. Plouffe, M. F. Para, and B. Hackman.** 1985. Aerosols containing *Legionella pneumophila* generated by shower heads and hot-water faucets. Appl. Environ. Microbiol. **50:**1128–1131.
5. **Bradley, R. A., and D. E. W. Schumann.** 1957. The comparison of the sensitivities of similar experiments: applications. Biometrics **13:**496–510.

5a. **Burge, H.** Personal communication.

6. **Burge, H. A., J. R. Boise, J. A. Rutherford, and W. R. Solomon.** 1977. Comparative recoveries of airborne fungus spores by viable and non-viable modes of volumetric collection. Mycopathologia **61:**27–33.
7. **Burge, H. A., M. Chatigny, J. Feeley, K. Kreiss, P. Morey, J. Otten, and K. Petersen.** 1987. Bioaerosols. Appl. Ind. Hyg. **5:**R10–R16.
8. **Burge, H. A., and W. R. Solomon.** 1987. Sampling and analysis of biological aerosols. Atmos. Environ. **21:**451–456.
9. **Castellan, R. M., S. A. Olenchock, K. B. Kinsley, and J. L. Hankinson.** 1987. Inhaled endotoxin and decreased spirometric values. N. Engl. J. Med. **317:**605–610.
10. **Chatigny, M. A.** 1983. Sampling airborne microorganisms, p. E1–E9. *In* P. J. Lioy (ed.), Air sampling instruments for evaluation of atmospheric contaminants. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio.
11. **Croft, W. A., B. B. Jarvis, and C. S. Yatawara.** 1986. Airborne outbreak of trichothecene toxicosis. Atmos. Environ. **20:**549–552.
12. **Flaherty, D. K., F. H. Deck, J. Cooper, K. Bishop, P. A. Winzenburger, L. R. Smith, L. Bynum, and W. B. Witmer.** 1984. Bacterial endotoxin isolated from a water spray humidification system as a putative agent of occupation-related lung disease. Infect. Immunol. **43:**206–212.
13. **Gravesen, S.** 1979. Fungi as a cause of allergic disease. Allergy (Copenhagen) **34:**135–154.
14. **Greene, V. W., D. Vesley, R. G. Bond, and G. S. Michaelsen.** 1962. Microbiological contamination of hospital air. Appl. Microbiol. **10:**561–566.
15. **Jacobs, R. R.** 1989. Airborne endotoxins: an association with occupational lung disease. Appl. Ind. Hyg. **4:**50–56.
16. **Lach, V.** 1985. Performance of the surface air system samplers. J. Hosp. Infect. **6:**102–107.
17. **Lembke, L. L., R. N. Kniseley, R. C. Van Nostrand, and M. D. Hale.** 1981. Precision of the all-glass impinger and the Andersen microbial impactor for air sampling in solid-waste handling facilities. Appl. Environ. Microbiol. **42:**222–225.
18. **Miller, J. D., A. M. Laflamme, Y. Sobol, P. Lafontaine, and R. Greenhalgh.** 1988. Fungi and fungal products in some Canadian houses. Int. Biodeterior. **24:**103–120.
19. **Reynolds, S. J., A. J. Striefel, and C. E. McJilton.** 1990. Elevated airborne concentrations of fungi in residential and office environments. Am. Ind. Hyg. Assoc. J. **51:**601–604.
20. **Samson, R. S.** 1985. Occurrence of moulds in modern living and working environments. Eur. J. Epidemiol. **1:**54–61.
21. **Sattar, S. A., and M. K. Ijaz.** 1987. Spread of viral infections by aerosols. Crit. Rev. Environ. Control **17:**89–131.
22. **Sayer, W. J., N. M. MacKnight, and H. W. Wilson.** 1972. Hospital airborne bacteria as estimated by the Andersen sampler versus the gravity settling plate. Am. J. Clin. Pathol. **58:**558–562.
23. **Sayer, W. J., D. B. Shean, and J. Ghosseiri.** 1969. Estimation of airborne fungal flora by the Andersen sampler versus the gravity settling plate. J. Allergy **44:**214–227.
24. **Seidler, R. A., and S. C. Hern.** 1988. EPA special report: the release of ice minus recombinant bacteria at California test sites. Environmental Research Laboratory, Corvallis, Oreg.
25. **Sorenson, W. G., D. G. Frazer, B. B. Jarvis, J. Simpson, and V. A. Robinson.** 1987. Trichothecene mycotoxins in aerosolized conidia of *Stachybotrys atra*. Appl. Environ. Microbiol. **53:**1370–1375.

25a. **Starks, T.** Personal communication.

26. **Stetzenbach, L. D.** 1992. Airborne microorganisms, p. 53–66. *In* J. Lederberg (ed.), Encyclopedia of microbiology. Academic Press, Harcourt Brace Joanovich Publishers, San Diego, Calif.
27. **Stetzenbach, L. D., S. C. Hern, and R. J. Seidler.** 1991. Field sampling design and experimental methods for the detection of airborne microorganisms, p. 543–555. *In* M. A. Levin, R. J. Seidler, and M. Rogul (ed.), Microbial ecology: principles, methods and applications. McGraw-Hill, Inc., New York.
28. **Thorne, P. S., M. S. Kiekhaefer, P. Whitten, and K. J. Donham.** 1992. Comparison of bioaerosol sampling methods in barns housing swine. Appl. Environ. Microbiol. **58:**2543–2551.
29. **Woodward, E. D., B. Friedlander, R. J. Lesher, W. Font, R. Kinsey, and F. T. Hearne.** 1988. Outbreak of hypersensitivity pneumonitis in an industrial setting. J. Am. Med. Assoc. **259:**1965–1969.
30. **Zeterberg, J. M.** 1973. A review of respiratory virology and the spread of virulent and possibly antigenic viruses via air conditioning systems. Ann. Allergy **31:**228–234.