A Comparison of Replicate Field Samples Collected with the Bi-Air, Air-O-Cell, and Graesby-Andersen N6 Bioaerosol Samplers

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INTRODUCTION

Background

Slit impaction samplers such as the Air-O-Cell (AOC) cassette are one of the most commonly used methods for collecting airborne fungal spores (Chen et al, 2004; Baxter et al, 2005). They are simple to use, easy to analyze, and cost effective. However, Chen et al (2004) noted that slit impaction samplers have several important limitations.

One limitation is their relatively high airflow rate, which is generally maintained in a narrow range. This may affect aspiration efficiency for spore clusters and larger spores; and limits the ability to vary sampling parameters to match field conditions and spore concentrations. Second, they are generally limited to the collection of short-term "grab" samples of 10 minutes or less. This limits their utility for collecting long-term (60 minutes or longer) time-weighted average (TWA) and/or personal samples. Third, their ability to retain spores is affected by particle size and spore morphology, resulting in variable retention rates for different spore types. In addition, the gap between the sampler inlet and the impaction surface results in an unbounded and diffuse sample trace (rectangular or curcular area on the collection medium in which the sample has been deposited), increasing the difficulty of detecting all the deposited spores (Aizenberg et al. 2000; Spurgeon 2006).

The desirable characteristics of a bioaerosol sampler have been described by several authors. Macher (1997) mentioned low airflow rate, low transmission losses, size-selective sampling efficiency, and ease of handling as desirable characteristics. Aizenberg et al. (2000) included low induced turbulence at the inlet, the use of conductive materials to minimize electrical effects, and uniform particle deposition on the collection medium as desirable characteristics. Feather and Chen (2003) listed a number of additional characteristics that were desirable in a bioaerosol sampler. These included small size; disposable collection medium; ability to collect area and personal samples; ability to collect both short- and long-term samples; applicability to a wide range of concentrations; high aspiration and collection efficiencies; simple analysis without sample transfer; and the ability to support a variety of analytical methods. McDevitt et al. (2005) also cited the ability to collect integrated airborne samples that were more representative of prolonged exposures.

Filter samplers have many of these desirable characteristics, and offer the potential for improved performance for the collection of airborne fungal spores. For example, McDevitt et al. (2005) described filter samplers as the preferred method for performing exposure assessments for bioaerosols. This was due to their high collection efficiency, long sampling times, the ability to collect personal samples, wide concentration range, and ability to be used with either high- or low-volume air pumps.

The Bi-Air (BA) filter cassette was specifically designed for the collection of airborne fungal spores, and has many of the desirable characteristics of a bioaerosol sampler (Spurgeon 2006). It also has a relatively small sample trace with well-defined boundaries; a design that produces duplicate sample traces; and the ability to vary capture velocity independently of airflow rate.

McGrath et al (1999) reported that complaint buildings frequently had high airborne concentrations of *Penicillium* species, while *Cladosporium* was usually dominant in buildings with few complaints. Similarly, Baxter et al (2005) concluded that indoor concentrations of airborne *Aspergillus/Penicillium* (*Asp/Pen*) like spores, as compared to other spore types, had the most utility in determining whether a building was contaminated with mold. These studies suggested that airborne concentrations of *Asp/Pen* like spores may be useful in differentiating clean from contaminated indoor environments. Therefore, concentrations of *Asp/Pen* like spores were used as the primary basis for comparing the relative performance of the AOC and BA cassettes.

In the study by Baxter et al (2005), the AOC samples were collected in moldy residential properties located in southern California. *Asp/Pen* spores were reported in 92 % of the samples, *Stachybotrys* in 16 % of the samples, and *Chaetomium* spores were not reported, suggesting they were either not detected or only detected in a small percentage of samples. The samples described in the current study were also collected in residential properties located in southern California. Therefore, the rank order for *Chaetomium* spores was expected to be similar in the two studies. Seventy-six BA samples collected in potentially contaminated residential properties and contained in an internal database were reviewed. *Asp/Pen* spores were detected in 96 % of the samples, *Chaetomium* in 47 %, and *Stachybotrys* in 45 % of the samples. *Chaetomium* was the second most frequently detected contaminant spore in the 76 BA samples reviewed, and it is commonly detected in BA samples collected in potentially contaminated indoor environments.

Foto et al. (2005) have also presented data collected with the AOC as well as other methods. In that study, airborne glucan and ergosterol concentrations were reported to be correlated to visible mold damage in the subject properties. However, neither spore counts nor the prevalence of airborne *Asp/Pen* in the AOC samples were related to the extent of visible mold damage. Although *Asp/Pen* spores comprised about 51 % of total spores, *Chaetomium* was again not reported in the AOC samples. Therefore, the *Chaetomium* spore was included in the current study to explore potential differences in the rank order of spores reported by slit-impaction and filter samplers.

A previous study compared the performance of the BA and the AOC for the collection of *Asp/Pen* spores under controlled conditions in a quiescent settling chamber (Spurgeon 2006). The settling chamber was representative of a well-mixed environment, whereas field investigations are typically performed under less controlled conditions. Second, spores were introduced into the chamber as single spores in a liquid suspension. In addition, comparisons were limited to a maximum *Asp/Pen* concentration of about 30,000 spores/m^3, while *Asp/Pen* concentrations of 10^6 spores/m^3 have been detected in BA samples during several residential investigations.

Although a number of comparative studies of bioaerosol samplers have been performed in the laboratory, only a limited number of comparisons based on field studies have been reported (Lee et al. 2004). However, field environments may contain clusters of spores as well as single spores, a wide variety of spore sizes and types may be encountered, and other confounding factors influencing sampler performance that may not be included in laboratory studies. Therefore, comparing the performance of bioaerosol samplers under actual field conditions is of practical importance.

Purpose

The purpose of this study was to compare the relative performance of the BA and the AOC for the collection of *Asp/Pen* and *Chaetomium* spores under actual field conditions. These comparisons were based on the collection of duplicate and/or replicate field samples during residential investigations. One objective of the study was to assess the effects of particle size and spore morphology on the retention of spores by the BA and the AOC. Differences in spore retention were evaluated based on the concentrations of *Asp/Pen* and *Chaetomium* spores detected in replicate field samples. A second objective of this study was to compare the BA and the AOC at *Asp/Pen* concentrations higher than those used in the previous chamber study (Spurgeon, 2006).

The BA produces two independent sample traces. If an elevated concentration of *Asp/Pen* spores is detected in the first sample trace, the second sample trace may be submitted for culturing. A third objective of the study was to compare the concentrations of culturable *Aspergillus* and *Penicillium* for duplicate and replicate field samples collected using the BA and the Graesby-Andersen N6 sampler.

METHODS

Sampling Conditions

The samples described in Table 1 were collected in the living room of a single family house during a mold restoration response. Although work was not being performed, several drying fans were operating in the room, which kept the air well-mixed during the sampling period. Three tripods arranged in a triangular pattern were used to collect six 5-minute replicate samples with both the BA and the AOC and 2-minute samples with the N6. One set of replicate samples was collected about every ten minutes during a 66 minute period. In contrast, the sample results contained in Tables 2 – 5 were collected in

undisturbed indoor environments typical of residential mold investigations.

Air-O-Cell Cassette

The airflow rate was calibrated to 15 lpm using a direct reading 60 mm rotameter (0-30 lpm). The AOC was attached to a tripod and positioned vertically at a height of five feet. A sample was then collected for five minutes. Following sample collection, the AOC cassette was opened and the adhesive strip was removed. A drop of liquid adhesive was placed on a glass slide, and the bottom of the sample slide was placed on the liquid adhesive. The sample was stained with lactophenol cotton blue, covered with a cover slip, and analyzed by light microscopy.

Bi-Air Cassette

The BA contained a mixed cellulose ester (MCE) filter (Millipore Corp, Bedford, MA) with a pore size of 1.2 um, which was supported by a cellulose pad. Samples were collected at about 1 lpm when using a Model 224-PCXR8 (SKC, Inc., Eighty Four, PA) low-volume pump with programmable start/stop features; and at airflow rates up to 3.6 lpm when using a high-volume pump. The BA was attached to the pump inlet in a vertical position using a short piece of tubing. The pump was placed on a table top, desk top, or other object when using a low-volume pump; and oriented vertically on a tripod when using a high-volume pump. Sample times were typically 60 minutes, but ranged from 50 to 90 minutes.

Following sample collection, the cassette was opened and the filter and pad were placed on a clean surface. The two sample traces were separated by cutting the filter in half using an Xacto Knife with a ½ inch blade. One sample trace was placed on a glass slide, and the slide inserted into a Quick Fix® acetone vaporizer (EMS, Charleston, SC) to collapse and clear the MCE filter. The rough surface of the MCE held the spores in place during handling and staining. The sample was then stained with lactophenol cotton blue, covered with a cover slip, and analyzed by light microscopy.

Graesby-Andersen N6 Impactor

A Graesby-Andersen single-stage N6 Impactor was disassembled and the component parts wiped with an alcohol swab. A media plate containing malt extract agar (MEA) was opened and inserted into the sampler, and it was reassembled. The N6 was attached to a tripod at a height of about five feet in a vertical orientation. A high-volume air sampling pump with a minimum capacity of 30 lpm was attached to the sampler, and the airflow rate was calibrated to 28.3 lpm using a 150 mm high precision rotameter (0-40 lpm) that had been calibrated with a Bios Dry Cal® primary standard. Samples were typically collected for two minutes as measured by a stopwatch.

Following sample collection, the sampler was opened, the media plate was covered and removed from the sampler, then sealed with masking tape. The sample was stored in an insulated container in the field, then refrigerated until it was express shipped to the

laboratory no later than the following day. The samples were cultured at 25 °C and analyzed by a microbiology laboratory which was a participant in the American Industrial Hygiene Association (AIHA) Environmmental Microbiology Laboratory Accreditation Program (EMLAP). Concentrations were reported as colony forming units per cubic meter (cfu/m^3).

Spore Counting Procedures

Fungal spores were identified and counted by bright field microscopy at 600X magnification. Analyses were performed using a Nikon Labophot-2 or Alphaphot PCM microscope (Nikon America, NY, NY) equipped with a 10x eyepiece and planachromat 10x, 20x and 60x objectives. Samples with the highest spore densities were analyzed by counting eight randomly selected fields of view. The estimated number of spores in a cluster were reported rather than reporting the cluster as a single colony forming unit (Palmgren et al. 1986; Eduard and Aalen 1988; Eduard et al. 1990).

The AOC and BA samples were analyzed using the same protocols commonly employed for the routine analysis of field samples. The AOC samples were analyzed by beginning the analysis near one end of the sample trace, but within the sample area. Every other traverse (area across the trace the width of the microscopic field of view) was analyzed until a maximum of eleven traverses had been analyzed, or about 22 % of the sample trace. The BA samples were analyzed by starting at the first traverse along one edge of the sample area, and analyzing every traverse until a maximum of ten traverses had been analyzed, or about 50 % of the sample. Analyzing 22 % of the AOC and 50 % of the BA sample traces required about the same amount of time, which was a practical constraint when analyzing multiple sets of field samples. In addition, Foto et al (2005) have reported that analyzing 25 % of the AOC sample trace was sufficient to achieve reproducible results.

RESULTS

The data in Table 1 describe the concentrations of *Asp/Pen* spores and culturable *Aspergillus* and *Penicillium* for six replicate airborne samples collected in a well-mixed residential environment. The culturable N6 and BA samples in Table 1 were submitted to the same microbiology laboratory. However, the N6 samples were reported as *A. penicillioides* while the BA samples were reported as *A. versicolor*. Therefore, the *Aspergillus* concentrations in Table 1 were not identified to species.

TABLE 1. Concentrations of total *Asp/Pen* spores (spores/m^3) and culturable *Aspergillus* and *Penicillium* species (sp.) (cfu/m^3) for six replicate samples collected in a well-mixed room with the BA, AOC and N6 samplers during a 66-minute period.

	Asp/Pen Spores		*Aspergillus* sp.		*Penicillium* sp.	
SAMPLER	**BA**	**AOC**	**BA**	**N6**	**BA**	**N6**
N	6	6	5	6	6	6
Min	8,000	3,400	1,100	2,400	1,100	2,100
Max	15,100	7,100	4,400	3,000	4,400	3,900
AVG	11,300	5,350	2,320	2,770	2,400	2,850
RSD (%)	28 %	27 %	63 %	8 %	51 %	25 %
Vol (l)	9	75	9	56	9	56

The airflow rates in Table 1were 3.6 lpm for the BA samples, 15 lpm for the AOC samples, and 28.3 lpm for the N6 samples. The sample volumes were 18 liters (9 l per trace) for the BA, 57 liters for the N6, and 75 liters for the AOC. About 50 % of one BA sample trace (10 traverses) and 22 % of the AOC trace (11 traverses) were analyzed for total spores. However, the relative standard deviations (RSD) for the BA and AOC data were 28 % and 27 %, respectively.

The average concentration of *Asp/Pen* spores for the BA samples in Table 1 was 11,300 spores/m^3 while the average concentration for the AOC samples was 5,350 spores/m^3, a ratio of 2.1. An ANOVA indicated the two sample means were statistically different ($F = 18$, $F_{crit} = 4.96$, $P = 0.0017$).

The culturable *Aspergillus* concentration in the second BA sample included in Table 1 was reported as below the limit of detection (LOD). However, the *Penicillium* concentration in the same sample was reported to be 1,700 cfu/m^3, which was consistent with the other samples. That sample result was considered to be anomalous, and was not included in the calculated parameters for *Aspergillus*. Except for this discrepancy, the average concentrations reported for the BA and the N6 were similar for both *Aspergillus* ($F = 0.29$, $F_{crit} = 4.96$, $P = 0.60$) and *Penicillium* ($F = 0.61$, $F_{crit} = 4.96$, $P = 0.45$).

The 19 duplicate samples described in Table 2 are concentrations of total culturable fungi collected with the N6 and the BA samplers during field investigations. The samples were divided into those with total concentrations less than and greater than 1,000 cfu/m^3 as measured by the N6. The 2-minute N6 samples were analyzed by direct plate count, while the 10-minute BA samples were analyzed by dilution plating.

TABLE 2. Comparison of concentrations of total culturable fungi (cfu/m^3) for duplicate samples collected with the N6 and the BA samplers; N6 concentrations less than and greater than 1,000 cfu/m^3.

	< 1,000 cfu/m^3		> 1,000 cfu/m^3	
Sampler	**N6 Sampler**	**BA Sampler**	**N6 Sampler**	**BA Sampler**
N	10	10	9	9
Min	180	570	954	570
Max	620	1,430	6,870	7,720
AVG	410	685	4,250	3,360

The coefficient of correlation (r) between the N6 and BA data for N6 concentrations less than 1,000 cfu/m^3 was 0.09, indicating little correlation. However, the r-value between the N6 and BA data for N6 concentrations greater than 1,000 cfu/m^3 was 0.76. An ANOVA performed on the nine samples with concentrations greater than 1,000 cfu/m^3 indicated the two means were similar ($F = 0.36$, $F_{crit} = 4.49$, $P = 0.56$).

The replicate BA and AOC samples in Table 3 were collected from eight residential projects. *Asp/Pen* spores were detected in all 27 of the BA and AOC samples in Table 3. The average *Asp/Pen* concentration detected with the BA (12,000 spores/m^3) exceeded the average concentration detected with the AOC (7,550 spores/m^3) by a factor of 1.6. When the data for paired samples were analyzed, the BA:AOC ratios for the 27 paired *Asp/Pen* samples ranged from 0.5 to 4.5, with an average of 2.0.

TABLE 3. Comparison of 27 replicate BA and AOC samples collected from eight residential projects; total *Asp/Pen* and *Chaetomium* spore concentrations in spores/m^3.

	Aspergillus/Penicillium			*Chaetomium*		
	BA	AOC	RATIO	BA	AOC	RATIO
N	27	27	27	20	16	24
MIN	700	500	0.5	30	60	0.3
MAX	51,400	43,500	4.5	3,700	6,100	89
AVG	12,000	7,550	2.0	910	880	8.6

Chaetomium spores were detected in a total of 24 of the 27 samples described in Table 3; and were detected in a total of 20 BA samples and 16 AOC samples. This spore was detected only by the BA in eight samples and only by the AOC in four samples. The ratios for the 24 individual samples were calculated by assigning a *Chaetomium* concentration of half the LOD to the four BA samples and eight AOC samples that were censored (concentration below the LOD). The average *Chaetomium* concentration detected with the BA (910 spores/m^3) was similar to the average concentration detected with the AOC (880 spores/m^3). However, when the data for paired samples were analyzed, the BA:AOC ratios for the 24 paired *Chaetomium* samples ranged from 0.3 to 89, with an average of 8.6.

The data in Table 4 are for four replicate samples that were collected during one project. The replicate 60-minute BA and 5-minute AOC samples were collected in the same rooms and during the same sampling period. Small *Asp/Pen* like spores (< 3 μm) and large clusters of *Asp/Pen* like spores (> 50 spores/cluster) were dominant in the BA samples, whereas single spores or small chains are more typically encountered in field samples. The average concentration of *Asp/Pen* like spores detected with the BA samples was 765,000 spores/m^3, which substantially exceeded the average concentration detected with the AOC samples (36,800 spores/m^3). The BA:AOC ratio of *Asp/Pen* spore concentrations ranged from 14 to 146, with an average ratio of 54.

TABLE 4. Total *Asp/Pen* and *Chaetomium* spore concentrations (spores/m^3) for four replicate BA and AOC samples collected in a residential property; small *Asp/Pen* spores and clusters of *Asp/Pen* spores dominant.

	Asp/Pen			*Chaetomium*	
ROOM	**BA**	**AOC**	**RATIO**	**BA**	**AOC**
Living Room	365,000	24,500	15	1,800	60
Kitchen	585,400	14,800	40	1,150	0
Bedroom	702,500	4,800	146	300	0
Bathroom	1,406,000	103,200	14	2,700	0
AVG	765,000	36,800	54	1,500	15
RSD	59 %	122 %	NA	68 %	NA

Chaetomium was detected in all four BA samples in Table 4, but was only detected in one AOC sample, and that was at the LOD. The average *Chaetomium* concentration for the BA samples was 1,500 spores/m^3, with an average concentration of 15 spores/m^3 for the AOC samples, resulting in a BA:AOC ratio for the average *Chaetomium* concentration of 100 for the four samples. In addition, the RSD for the *Asp/Pen* concentrations was 59 % for the BA and 122 % for the AOC, although an autocorrelation effect based on sample location was expected to increase the variance for both samplers.

The data in Table 5 were collected in one of 20 apartments that were sampled in a complex. Replicate 60-minute BA and 5-minute AOC samples were collected in the three bedrooms. However, the comparison was limited by the absence of AOC samples in the master bathroom and the kitchen. The *Asp/Pen* concentrations detected with the five 60-minute BA samples resulted in a concentration gradient, with the highest concentration in the master bathroom, intermediate concentrations in the hall bedrooms, and the lowest concentration in the kitchen.

TABLE 5. Total *Asp/Pen* spore concentrations (spores/m^3) collected with 60-minute BA and 5-minute AOC samples in a three-bedroom apartment during the same time period.

ROOM	BA (60 Minute)	AOC (5 Minute)
Mstr Bathroom	84,900	NA
Mstr Bedroom	50,700	6,700
Hall Bedroom 1	45,300	91,500
Hall Bedroom 2	20,200	43,500
Kitchen	15,200	NA
AVG (n = 3)	38,700	47,200
RSD (%)	42 %	90 %

The floor plan of the apartment is illustrated in Figure 1. The BA samples clearly indicated the master bathroom was the locus of contamination. Although the 5-minute AOC samples were only collected in the three bedrooms, a similar concentration gradient was not apparent for the AOC samples. The RSD's for for the three bedroom samples were 42 % for the BA samples and 90 % for the AOC samples. However, the BA data appeared to be autocorrelated based on sample location, which would exaggerate the variance and overestimate the RSD.

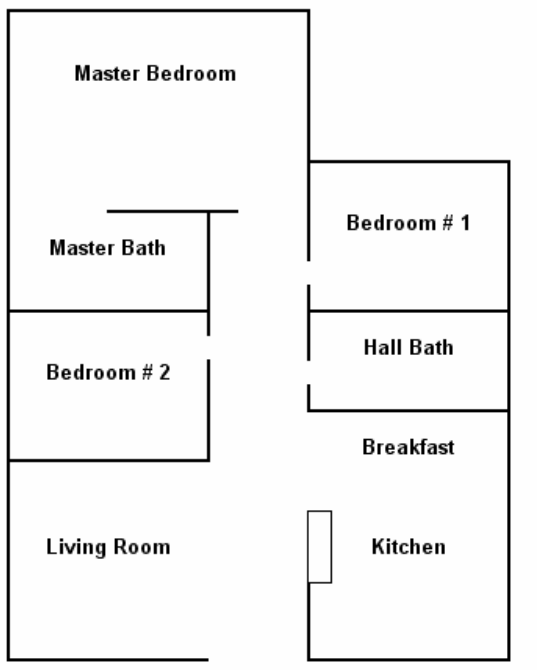

Figure 1. Floor plan of the apartment from which the Table 5 data were collected.

DISCUSSION

Table 1

The average *Asp/Pen* concentration in Table 1 that was detected with the BA in a well-mixed room was 2.1 times the average concentration detected with the AOC. This ratio was equivalent to that obtained in a previous study conducted in a well-mixed settling chamber (Spurgeon 2006). Therefore, the results for *Asp/Pen* spores collected with the BA and the AOC were consistent with those of the previous chamber study. In addition, the sample size was sufficient for the data to be statistically significant.

The BA spore data in the referenced chamber study were collected for 30 minutes at an airflow rate of 1.0 lpm, while the BA spore data in Table 1 were collected for 5 minutes at an airflow rate of 3.6 lpm. The actual sample volume analyzed for total spores with the BA was a relatively low 4.5 liters (l), whereas 16.5 l were analyzed for the AOC. However, the RSD values for *Asp/Pen* spores were similar for both the BA and the AOC (28 % & 27 %). In addition, the RSD values in Table 1 were similar to those obtained in the referenced chamber study (27 % & 29 %). Therefore, the variations in sampling time, airflow rate, and sample volume in the two studies did not have a noticeable effect on the comparability of the data when the samples were collected in a well-mixed environment.

However, the RSD for culturable fungi varied over a wide range. The RSD values for the N6 were a relatively low 8 % for *Aspergillus* and 25 % for *Penicillium*, while the respective values for the BA were 63 % and 51 %. The larger RSD values for the BA data were attributed to the use of dilution plating methods.

The similarity of the data obtained in the well-mixed room described in Table 1 and the settling chamber study suggested that conditions in the settling chamber study were similar to a well-mixed indoor environment. However, the conditions in the well-mixed room were artificially created by drying blowers, and the environment during a typical field investigation is not as well mixed. Therefore, such well-mixed conditions were not representative of those typically encountered during field investigations. This issue was explored by comparing the results for replicate field samples collected under typical field conditions.

The data in Table 1 suggest that the BA might be useful for measuring the airborne concentrations of culturable fungi when the N6 or similar samplers cannot be used. For example, the BA may be useful when high spore concentrations are expected, for collecting TWA samples, and for measuring personal dose. However, the small sample size in Table 1 was a limitation. A similar comparison that included a larger sample size and extended-period samples would be beneficial.

One advantage of the BA for culturable fungi was that, following spore analysis, those samples with elevated spore counts could be identified for culturing. Those limited samples could then be cultured using the various media appropriate for the detected spore types. The data in Table 1 suggest that the first BA sample trace could be used to

quantitatively evaluate total spore concentrations; while the second sample trace could be used for the qualitative, and possibly quantitative analysis of *Asp/Pen* and other spore types.

Table 2

A second advantage was the wide concentration range provided by the dilution plating method. For example, one field sample with an N6 concentration of 5,620 cfu/m^3 and a BA concentration of 26,000 cfu/m^3 was not included in Table 2. However, in addition to being more variable than the N6, dilution plating did not have the ability to discriminate between low concentrations. Although the N6 data were evenly distributed within the low concentration range in Table 2, eight of the 10 BA concentrations were reported as 570 cfu/m^3. It was apparently difficult to perform a quantitative analysis using dilution plating methods on those BA samples with total fungal concentrations less than 1,000 cfu/m^3. However, BA field samples with *Asp/Pen* spore concentrations of less than about 2,000 spores/m^3 are generally not submitted for culturing. The primary interest is in those samples that have elevated spore counts; and, a sufficient number of viable spores must be present for culturing.

The nine samples in Table 2 with total fungal concentrations greater than 1,000 cfu/m^3 as measured with the N6 were correlated with the BA samples; and, the mean concentrations were similar for the two samplers. In addition, total fungal concentrations in a larger set of 51 replicate BA and N6 samples were also correlated with $r = 0.80$. These data, and those in Table 1, suggest the BA could be useful for quantitating culturable fungi when the samples were collected from potentially contaminated environments.

The culturable samples discussed in Table 1 and Table 2 were collected over relatively short sampling periods of 10 minutes or less. However, the recovery of culturable fungi using a filter cassette was reported to be suitable for collection times of up to two hours, and could be used for the collection of both area and personal samples (Palmgren et al. 1986). The effect of sampling time on the recovery of culturablefungi and bacteria for filter samples collected at a composting facility has also been reported (Durand et al. 2002). Sampling times of 6 hours at an airflow rate of 2 lpm did not have a detectable effect on the recovery of *Aspergillus* and *Penicillium* species, which were the dominant fungi. Therefore, filter media have been used to collect culturable fungi over periods of time substantially longer than those reported in Tables 1 and 2.

Table 3

The *Asp/Pen* and *Chaetomium* concentrations from 27 replicate BA and AOC field samples are described in Table 3. The replicate samples were collected from eight residential projects, with either three or four replicate samples collected for each project. The ratio of 2.0 for the *Asp/Pen* spores in Table 3 was equivalent to the ratio obtained in Table 1, as well as in controlled studies (Aizenberg et al. 2000; Spurgeon 2006). Therefore, the average performance of the BA and the AOC in controlled tests was

similar to that for the field samples described in Table 3 for *Asp/Pen* spores. This result implied that the test conditions employed in those studies were representative of typical field conditions.

Other slit-impaction cassettes may also have similar ratios for *Asp/Pen* spores. For example, a limited comparison (n = 4) of *Asp/Pen* spore concentrations collected in replicate BA and Allergenco D cassette samples (EMS, Charleston, SC) resulted in a BA:AD ratio of 1.4 to 1.75 with an average of 1.6.

However, the *Chaetomium* concentrations detected in the BA samples in Table 3, on average, exceeded those concentrations detected in the AOC samples by a factor of 8.6; with a BA:AOC ratio of 100 for the data in Table 4. *Chaetomium* is a relatively large spore (8-12 μm), has a smooth surface, and has a sub-spheroid shape similar to an over-inflated football (Watanabe 1994). Therefore, particle bounce could be an important factor in the retention of *Chaetomium* spores. In contrast, *Stachybotrys chartarum* also has larger spores that are in a size range somewhat similar to *Chaetomium*, but these spores have a rough surface and a larger contact area. Since the retention efficiencies for *S. chartarum* spores were similar for the BA and the AOC in the previous chamber study (Spurgeon, 2006), the substantial difference in retention efficiency for *Chaetomium* was attributed primarily to spore morphology (particle bounce) rather than differences in aspiration efficiency.

Table 4

The data presented in Table 4 are for four replicate BA and AOC samples collected from a single project in which small *Asp/Pen* spores and spore clusters were dominant. The aggregation of spores on filter samples has been noted (Eduard and Aalen 1988; Eduard et al. 1990). Although the level of aggregation varied by spore type, somewhat fewer than 50 % of spores were detected as aggregates in those studies. Therefore, clusters may represent a substantial percentage of the total *Asp/Pen* spore count in some indoor environments.

The average BA:AOC ratios in Table 4 were 54 for *Asp/Pen* spores and 100 for *Chaetomium* spores. The wide variation in average concentrations between the two samplers could have been due to an unintentional disturbance of the environment during the sampling period. However, any such disturbance would have had to affect all four rooms simultaneously. Such a disturbance was not noted at the time of the investigation, but could have occurred.

The data presented in Table 1 implied an approximately constant BA:AOC ratio of about 2.0 for *Asp/Pen* concentrations in well-mixed indoor environments when spores with diameters of about 3 μm or larger were dominant. However, the ratios in Table 4 indicated a substantial difference in relative performance between the two samplers in that particular field environment. More importantly, these data suggest that the BA:AOC ratio for *Asp/Pen* spores may not be a constant in all indoor environments.

As for the data in Table 3, the difference in performance between the two samplers reflected in the Table 4 data was attributed to differences in aspiration efficiency (spores v. clusters), collection efficiency (*Asp/Pen*) and spore morphology (*Chaetomium*). It is not unusual to detect *Asp/Pen* like spores with a diameter of about 2 microns (μm) in BA samples, although similar spores have not been observed in AOC samples. These spores, often appearing in chains, made up a substantial fraction of the *Asp/Pen* spores detected in the BA samples. The larger *Asp/Pen* spores had a smooth surface, were typically 3-6 μm in diameter, and were predominantly present as clusters. The *Chaetomium* spores were larger (8-12 μm), had a smooth surface, and were sub-spheroid in shape. Therefore, particle bounce was assumed to be the primary reason for the relatively low detection rates for *Chaetomium* with the AOC.

Both aspiration efficiency and particle bounce may have affected the retention of *Asp/Pen* spores present as clusters, since smaller *Asp/Pen* clusters were observed in the AOC samples. The leading spores in a large cluster would encounter an adhesive surface. As the cluster broke apart upon impact, the trailing spores in the cluster may have encountered the previously retained spores rather than an adhesive surface. This may have caused the trailing spores to rebound into an air stream that was moving parallel to the collection medium. This mechanism could also be operative in the nasopharyngeal region of the respiratory tract, transforming some fraction of inhalable clusters into respirable spores.

Baxter et al indicated that for AOC samples, *Asp/Pen* spores were one of the more frequently encountered spores in contaminated indoor environments (Baxter et al. 2005). The minimum *Asp/Pen* concentration detected in the AOC samples in Table 4 was 4,800 spores/m^3. Assuming this concentration would be sufficient to classify those rooms as contaminated, both the BA and the AOC were equally capable of detecting the presence of airborne *Asp/Pen* spores and classifying that indoor environment as contaminated.

However, *Asp/Pen* spores less than about 3 μm in diameter, spore clusters, and spores similar in morphology to *Chaetomium* may be detected with less frequency by slit impaction samplers. For example, a limited comparison (n = 4) of *Asp/Pen* spore concentrations collected in replicate BA and Allergenco D cassette (EMS, Charleston, SC) samples resulted in a BA:AD ratio of 1.4 to 1.75 with an average of 1.6. Therefore, the data for *Asp/Pen* clusters and *Chaetomium* in Table 3 suggest the AOC, and possibly other slit impaction samplers, may not be suitable for performing risk assessments on exposed occupants.

Previous studies have had difficulty establishing a strong association between airborne fungal spore concentrations and occupant health effects (Committee on Damp Indoor Spaces and Health 2004). This lack of association may have been influenced by the type of samplers selected to collect the data. Indoor air quality investigators perform both exposure assessments and risk assessments. These two broad categories of activities often require the use of different methods. In addition, they may also require a different

quality of field data. The quality of data resulting from the use of slit-impaction samplers may be adequate for performing an exposure assessment (determining if an exposure existed), but may not be adequate for performing a risk assessment (assessing the potential risk to the occupant), where the average exposure as well as the maximum (95^{th} percentile exposure, for example) may be of concern.

Table 5

The longer sampling times possible when using filter samplers may offer a practical advantage for the collection of bioaerosol samples in field investigations (Palmgren et al. 1986; Aivenberg et al. 2000; Durand et al. 2002; McDevitt et al. 2005). Heterogeneous indoor environments are expected to experience localized variations in concentration over short time periods. Therefore, longer sampling times would be expected to provide a better estimate of average concentrations, and may be an important influence on data quality when sampling for bioaerosols.

The data in Table 5 describe the sample results for an apartment in which the ceiling in the master bathroom had collapsed due to a water intrusion from the upstairs unit. The apartment was arranged in a typical fashion as illustrated in Figure 1, with the master bedroom at the end of a hall, master bathroom opening into the master bedroom, bedroom 1 closest to the master bedroom, and the kitchen and living room occupying the front of the apartment.

Because of the high spore concentrations detected, these data provide a clear example of the potential influence of sampling time on the quality of field data. The RSD values for the replicate three-sample data sets collected in the 20 apartments that were sampled varied from 15 % to 50 % for the BA and 80 % to 150 % for the AOC. The larger RSD values for the BA, as illustrated in Table 5, occurred when the data were autocorrelated due to sampling location.

The BA data suggested the master bathroom was the origin of the airborne *Asp/Pen* spores within the apartment. The concentration gradient from the master bathroom to the kitchen was interpreted as diffusion from a point source. The data in Table 5 illustrate the generally accepted concept that longer sampling times provide a better estimate of the average concentration as compared to short-tem grab samples. The increased precision of the 60-minute BA samples resulted in data that were consistent with the incident history, while the short-term AOC samples were less consistent with the known history. However, this effect was probably only observed due to the high average concentrations present in the apartment.

SUMMARY

Many of the comparisons in this study were based on small sample sizes. However, the results were consistent with previous studies, which suggested that the reported results may be confirmed by more extensive studies. However, the lack of sample size was counterbalanced by limiting the comparisons to replicate and/or duplicate samples.

The average concentration of *Asp/Pen* spores for six duplicate samples collected in a well-mixed room indicated a BA:AOC concentration ratio of 2.1. This was similar to the expected ratio of about 2 based on previous studies conducted in controlled environments. In addition, the two sample means were statistically different. A BA:AOC ratio of 2 for *Asp/Pen* spores was also obtained when replicate field samples were compared. However, when small Asp/Pen spores and clusters of *Asp/Pen* spores were dominant, the average BA:AOC ratio of *Asp/Pen* spore concentrations ranged from 14 to 146 with an average of 54.

The *Chaetomium* spore, which has a morphology indicating its collection efficiency may be reduced by particle bounce in impaction samplers, was also under-detected by the AOC in comparison to replicate BA samples. The average BA concentration of *Chaetomium* exceeded the average AOC concentration by a factor of 8.6. A ratio of 100 was the highest BA:AOC ratio detected for a series of four replicate samples. In addition, the low frequency of detection of *Chaetomium* in AOC samples reported in previous studies by Baxter et al (2005) and Foto et al (2005) was consistent with the results of the current study. The results for *Asp/Pen* clusters and *Chaetomium* spores suggested that the AOC, and possibly similar slit impaction samplers, may substantially under-report average spore concentrations under some field conditions. Therefore, the use of such samplers to perform occupant risk assessments should be validated.

The average concentrations for culturable *Asp* and *Pen* were similar for six duplicate BA and N6 samples collected in a well-mixed room. In addition, the concentrations of total culturable fungi reported for short-term duplicate field samples collected with the BA and N6 were moderately correlated ($r = 0.76$), and the means were similar. In addition, the 10-minute BA samples were able to detect concentrations over a wider range compared to the 2-minute N6 samples. These results suggested that the BA could be used to collect culturable fungi when the N6 or similar samplers could not be used.

The BA may be preferable when collecting TWA samples, when ultra low spore concentrations have to be detected (clean rooms), to measure personal dose (health effects), or in environments where very high fungal concentrations were anticipated (agriculture, mold remediations). The AOC appeared to be suitable for detecting the presence of airborne contaminant fungal spores in indoor environments. However, the data for *Asp/Pen* clusters and *Chaetomium* suggest the AOC, and possibly other slit impaction samplers, may not be suitable for performing risk assessments on exposed occupants. Therefore, filter samplers may be preferable for evaluating associations between airborne fungal concentrations and potential health effects.

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