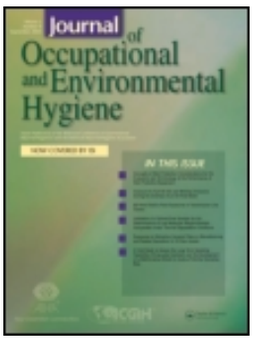

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Field Evaluation of a Personal, Bioaerosol Cyclone Sampler

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A personal cyclone sampler (cyclone) was operated continuously alongside a 25-mm filter sampler (filter), a slit impactor (Burkard slide), and a high-volume cyclone sampler (Burkard cyclone) at an outdoor location with abundant naturally occurring fungi ($N = 30$; sampling time: 12.5 ± 2.3 hr). Air concentrations (spore m^{-3}) of 28 fungal groups were determined for all samplers by microscopy. Cyclone performance was judged using various indices to determine if it agreed with the other samplers in determination of the frequencies with which the fungal groups were observed, as well as their proportions of the total air concentration. Fungal diversity estimates were similar for all samplers and in the range of what has been reported nationally, i.e., observation of 9–11 equal groups per sample, but spore concentration dominated by 2–3 groups. Plots of paired cyclone:comparison sampler ratios against average concentrations identified biases. For example, ratios were correlated with concentration and there was greater uncertainty at lower concentrations. Mean ratios for cyclone:filter comparisons were not significantly different from one for ascospores, Aspergillus-Penicillium *spp., basidiospores,* Cladosporium *spp., or total spore* m^{-3}. *However, agreement was less consistent with the Burkard slide (0.74, 1.12, 0.91, 1.09, and 0.92, respectively) and the Burkard cyclone (2.31, 1.62, 1.43, 1.91, and 1.33, respectively). Concentrations of cell equivalent* m^{-3} *also were determined for the filter and two cyclone samples by polymerase chain reaction. Cell equivalents for* Aspergillus fumigatus *and* Penicillium brevicompactum *were compared with* Aspergillus-Penicillium *spp. spores, and* Cladosporium cladosporioides *and* Cladosporium herbarum *cell equivalents were compared with* Cladosporium *spp. spores. Cell equivalent:spore ratios below one for* A. fumigatus *and* P. brevicompactum *indicated that these species comprised smaller factions of total spores or were collected less efficiently than the larger* C. cladosporioides *and* C. herbarum *spores. The personal cyclone was shown to be suitable for collection of ambient airborne fungal spores and for analysis by microscopy and polymerase chain reaction.*

Keywords cyclone sampler, fungal biodiversity, method comparison, outdoor air, sampler performance

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INTRODUCTION

Conventional methods for the measurement and identification of ambient airborne fungal spores rely on time-consuming and labor-intensive microscope and culture assays, and over the past decade, molecular techniques such as polymerase chain reaction (PCR) have been recognized as alternative and supplementary options for the detection of airborne fungi.[1–5] While acknowledging recent advances in the use of molecular methods to study fungal diversity, there still are many advantages to classical methods, i.e., reliance on the direct observation of fungi rather than measurement of fungal allergens or DNA.[6] For example, fungi recognizable by microscopy or culture isolation will be undetected by immunoassay or PCR if the researchers did not determine in advance to look for those species. On the other hand, it may be possible with molecular methods to detect specific fungi present at concentrations too low to be observed with other assays.[5] The performance of a personal aerosol sampler based on cyclone principles, which uses a 1.5-mL microcentrifuge tube (MCT) as a particle receptacle, has been evaluated for collection of fluorescently tagged polystyrene latex particles and polymer microspheres.[7] In addition to being easy to use, simple to fabricate, and inexpensive, long-term samples are possible with the cyclone, there is no sample transfer loss, particle recovery is high, and multiple analyses can be performed on each sample. Chamber tests demonstrated good agreement between the cyclone and other samplers for collection of single-cell, laboratory-generated aerosols of *Aspergillus versicolor* and *Scopulariopsis brevicaulis* over wide concentration ranges, with sampling times from 5 min to 5 hr and sample analysis by microscopy, culture, or PCR.[8]

Here, the suitability of the personal cyclone for collection of ambient, naturally generated fungal spores was examined through simultaneous, side-by-side comparisons with the

previously tested filter sampler and two devices widely used in aerobiological research that were too large to evaluate in the chamber study. The 30 samples were collected at a location with abundant airborne fungal spores on 33 days over a 52-day period (March–May) (collection time: 7–19 hr; one test per day with the exception of three samples that spanned 2 days because of interruption by rain). Collected spores were identified by microscopy and measurement of specific DNA sequences for the four most abundant species for which PCR analysis was available.

The goal of this study was to evaluate the suitability of the cyclone for operation outdoors and for analysis of ambient fungi by microscopy and PCR. Bioaerosol data is recognized to be largely qualitative in nature.[9] Therefore, cyclone performance was judged as the ability to describe the fungal community structure and to measure air concentration relative to three reference samplers. Reports of fungal biodiversity in indoor and outdoor air have used the number of different fungal groups (species richness) as the basis for judging biodiversity.[1,10–12] For this study, widely used richness, biodiversity, evenness, and similarity indices were calculated from microscopy data. A method to quantify the variability in the between-sampler and between-analysis agreement also was used to identify biases, measurements that might be outliers, and relationships that were dependent on the magnitudes of the measurements.[13]

MATERIALS AND METHODS

Sample Collection

Air Samplers

Table I lists the four bioaerosol samplers that were compared, their airflow rates, d_{50} cut points, collection media, and analyses. The capture of airborne particles by filtration is the most common method of aerosol sampling.[15] The slit impactor and two cyclone samplers collect spores by inertial impaction of particles in an airstream that is directed against a collection surface perpendicular or tangential to the direction of air movement.[16] The cyclone and filter samplers (NIOSH, Morgantown, W.Va.) were operated from one pump (model SP-280; Air Diagnostics and Engineering, Harrison, Maine) using a sampling manifold and flow-regulation valves. The personal cyclone sampler, with a 2-mm wide inlet, was operated at an airflow rate of 4.0 ± 0.05 L min^{1}, as measured continuously with an in-line mass flow meter (model 4140; TSI Incorporated, Shoreview, Minn.). The filter holder, with an 8-mm wide, 75-mm long sharp-edged probe; perforated stainless steel support screen; and 25-mm, 8.0-μm pore, cellulose ester membrane support filter (type SC filter; Millipore, Billerica, Mass.), also was operated at 4.0 ± 0.05 L min^{1}, measured continuously as above. Samples were collected on polycarbonate, capillary pore, membrane filters (25-mm, 0.8-μm pore, type ATTP filters; Millipore).

The Burkard Cyclone Sampler for Airborne Particles (C90M; Burkard Manufacturing Co., Hertfordshire, UK) was operated at ~16.6 L min^{1} with its internal air mover (first 6 of 30 samples) or an external pump (model 1532-107-G557X; GAST Manufacturing Corp., Benton Harbor, Mich.) (last 24 samples). If the pump was used, the average of the initial and final flow measurements was taken as the airflow rate (16.4 ± 0.52 L min^{1}). Conical, screw cap, 1.5-mL, MCTs (PGC Scientific, Frederick, Md.) were used with both cyclone samplers.[7]

The Burkard Continuous Recording Air Sampler was operated with its internal air mover; the default airflow rate of 10 L min^{1} was assumed because the unit had been calibrated prior to use in this study (personal communication, S. Katherine Hammond, University of California at Berkeley, September 2005). A 76- × 25- × 1-mm glass slide with petroleum jelly coating[17] advanced beneath the sampler's inlet at a rate of 2 mm hour^{-1}. All samplers were sanitized by wiping with ethanol after use.

Sampling Site and Time

The sampling site in Berkeley, California, was adjacent to a small park with abundant native and cultivated vegetation. Naturally occurring fungal spores were collected at ~2 m above ground from a 1.2- × 1.4-m platform (Figure 1) over a 9-week period (March 26–May 16, 2005). The cyclone and filter samplers were mounted with their inlets aligned horizontally, 12 cm apart. The two higher flow rate Burkard

TABLE I. Samplers Used in Field Comparison Tests

Air Sampler	Airflow Rate (L min^{-1})	d_{50} (μm)[A]	Collection Medium	Fungal Measurement[B]
Test bioaerosol sampler				
Personal cyclone (cyclone)	4.0	1.5	1.5-mL microcentrifuge tube	Spore, CFU, CE
Reference bioaerosol samplers				
Filter holder (filter)	4.0	—-	25-mm, 0.8 μm membrane filter	Spore, CFU, CE
Slit impactor (Burkard slide)	10.0	5.2	25-mm × 75-mm glass slide	Spore
Large-volume cyclone (Burkard cyclone)	16.6	1.2	1.5-mL microcentrifuge tube	Spore, CFU, CE

[A] Refs. 7,14.

[B] All fungi were identified for microscopy (spore) and culture (CFU), but only *A. fumigatus, C. cladosporioides, C. herbarum,* and *P. brevicompactum* were measured by PCR (CE).

TABLE II. Information on Which Concentrations Were Calculated for Four Air Samplers and Two Analyses

Sampler[A] (mean sample volume, coefficient of variation)	Fraction of Sample Analyzed	Mean Count (minimum–maximum)
	Microscopy (spore)	
Cyclone (3.0 m^3, 21%)	5%	Individual group: 11 (1–895) *Aspergillus-Penicillium* spp.: 15 (2–48) *Cladosporium* spp.: 26 (4–107) Total: 335 (69–949)
Filter (3.0 m^3, 21%)	5%	Individual group: 14 (1–828) *Aspergillus-Penicillium* spp.: 22 (4–63) *Cladosporium* spp.: 64 (1–384) Total: 444 (54–1113)
Burkard slide (7.4 m^3, 20%)	2.4%	Individual group: 22 (1–1059) *Aspergillus-Penicillium* spp.: 10 (1–27) *Cladosporium* spp.: 16 (3–72) Total: 669 (381–1230)
Burkard cyclone (12.3 m^3 , 22%)	5%	Individual group: 7 (1–411) *Aspergillus-Penicillium* spp.: 18 (2–83) *Cladosporium* spp.: 32 (1–227) Total: 208 (71–553)
	PCR (CE)	
Cyclone (3.0 m^3, 21%)	50%	*A. fumigatus*: 94 (4–490) *P. brevicompactum*: 50 (<5–260) *C. cladosporioides*: 1245 (13–3900) *C. herbarum*: 330 (2–1700)
Filter (3.0 m^3, 21%)	50%	*A. fumigatus*: 133 (<5–1000) *P. brevicompactum*: 89 (<18–790) *C. cladosporioides*: 1637 (180–5100) *C. herbarum*: 406 (15–2200)
Burkard cyclone (12.3 m^3, 22%)	50%	*A. fumigatus*: 61 (<2–210) *P. brevicompactum*: 245 (<6–5600) *C. cladosporioides*: 678 (7–8100) *C. herbarum*: 140 (<1–1800)

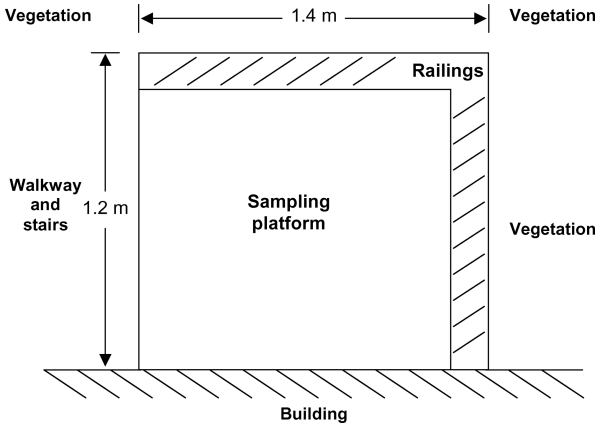

FIGURE 1. Diagram of the sampling platform. The filter and cyclone samplers were paired alternately on one railing and the Burkard slide and cyclone samplers on the other.

slide and cyclone samplers sat on one of two railings with the sampling inlets aligned, 40 cm apart. The inlets of the latter samplers were 15 cm below and facing 90° from the cyclone and filter samplers to avoid interference. The positions of the higher and lower flow rate samplers varied to allow as many permutations of sampler placement as possible during 30 trials.

All samplers were operated simultaneously for the entire sampling period, which ranged from 7 hr, 28 min to 19 hr, 21 min (mean: 12 hr, 26 min; SD: 2 hr, 20 min). Table II lists the air volumes for the different samplers. Sampling time increased over the study period to improve sensitivity (i.e., ability to detect spores present in low concentrations) as well as the reliability and relative precision of the measurements (i.e., concentration estimates based on detection of at least 10 spores per fungal group per sample). Sample collection started as early as 5:30 a.m. (median: 7:15 a.m.) and ended as late as 8:30 p.m. (median: 7:22 p.m.) as weather permitted. Samples were collected on all days of the week but most often on Friday,

Saturday, and Sunday (20%, 23%, and 17%, respectively) and least often on Wednesday (3%).

Sample Analysis

All analyses were performed by a lab accredited by AIHA for environmental microbiology and licensed by the U.S. Environmental Protection Agency for its PCR procedure. Slides, filters, and MCTs were held at room temperature until delivered overnight to the laboratory within 25 days of collection. Throughout this paper the term spore refers to all fungal propagules that were observed in air samples, i.e., spores and conidia (asexual spores).

Spores were recovered from the cyclone MCTs and filters by washing (final sample volume: 1 mL).[8] Recovery efficiencies were determined for 10 μL of a known spore suspension pipetted in triplicate into MCTs or onto filters (average efficiency = 88% and 90%, respectively). Table II shows the fractions of samples analyzed for microscopy and PCR and the number of spores or cell equivalents (CE) from which air concentration was determined.

Microscopy

Spores were counted by light microscopy at 600× magnification for all samples. One horizontal traverse of the 14-mm wide spore deposit on the Burkard slides was examined (length: 15–35 mm). Spores were counted for 50 μL of the filter and two cyclone spore suspensions, which had been spread on a glass slide with an imprinted counting grid and allowed to dry on a slide warmer for 5 min.

No spores were observed for three blank filters and three MCTs; average relative percent differences for total spores for three pairs of side-by-side, duplicate cyclone, filter, and Burkard slide samples were 15%, 27%, and 20%, respectively. The detection limit for microscopy was one spore (cyclone, filter, Burkard slide, and Burkard cyclone: 7, 7, 6, and 2 spore m^{-3}, respectively), and quantitation limits were 10 times the detection limits. The previously determined coefficient of variation (CV) for microscopy was 3%.

Culture

To identify fungal groups for PCR analysis, 100 μL (10%) of the spore suspensions for seven cyclone, filter, and Burkard cyclone samples were cultured on malt extract agar that was incubated at 25°C for 7 days. The detection limit for culture was one colony-forming unit (CFU) (cyclone, filter, and Burkard cyclone: 3, 3, and 1 CFU m^{-3}, respectively), and quantitation limits were ten times the detection limits. The previously determined CV for culture was 16%.

Sixteen fungi were identified by culture: *Acremonium* sp., *Acremonium strictum, Alternaria alternata, Aspergillus fumigatus, Aspergillus niger, Aspergillus sydowii, Aspergillus versicolor, Aureobasidium pullulans, Cladosporium* sp., *Cladosporium cladosporioides, Epicoccum nigrum, Penicillium* sp., *Penicillium brevicompactum, Penicillium chrysogenum, Scopulariopsis* sp., and *Trichoderma harzianum*. Concentrations for the 16 individual fungal groups for the cyclone, filter, and Burkard cyclone samples were calculated from mean (range) counts of <1 (1–13), <1 (1–6), and 1 (1–79) CFUs, respectively; total fungi: 7 (2–20), 8 (2–15), and 32 (1–168) CFUs, respectively.

PCR

Species-specific CE were determined using quantitative real-time PCR (qPCR)[18,19] for 500 μL of the filter and two cyclone samples. Cell suspensions were extracted by a rapid bead-milling method.[20] CE were determined from standard calibration curves based on counting spores in suspension. An internal reference was introduced prior to DNA extraction to account for extraction efficiency and PCR inhibition.[18,19] All primer and probe sequences used in the assays are available at the website: www.epa.gov/nerlcwww/moldtech.htm.

The 21 cultured samples were assayed for 22 specific DNA sequences: *Acremonium strictum, Alternaria alternata, Aspergillus fumigatus, Aspergillus niger*, ***Aspergillus ochraceus***, ***Aspergillus sydowii***, ***Aspergillus ustus,*** *Aspergillus versicolor, Aureobasidium pullulans,* ***Chaetomium globosum***, *Cladosporium cladosporioides, Cladosporium herbarum,* ***Cladosporium sphaerospermum,*** *Epicoccum nigrum, Penicillium brevicompactum, Penicillium chrysogenum, Penicillium variabile,* ***Scopulariopsis brevicaulis/fusca,*** *Scopulariopsis chartarum,* ***Stachybotrys chartarum,*** *Trichoderma viride/koningii, and* ***Ulocladium botrytis*** (eight bolded groups not detected). Based on these findings, *A. fumigatus, C. cladosporioides, C. herbarum*, and *P. brevicompactum* were chosen for PCR analysis of the remaining 69 samples because these four species were the most abundant fungi for which DNA sequences and genus-level spore count data were available. *A. fumigatus* and *P. brevicompactum* produce roughly spherical spores with dimensions of 2.5–3.0 and 3.0–4.5 μm, respectively.[21] Spores of *C. cladosporioides* and *C. herbarum* are ellipsoidal, 3–7 × 1–5 and 5.5–13 × 4–6 μm, respectively.[21]

PCR detection limits for individual samples and target sequences can differ due to variability in DNA extraction, the chemical composition of the samples, or differences in amplification efficiencies.[18] No samples were below detection for *C. cladosporioides*, but four Burkard cyclone samples were below detection for *C. herbarum* (<1 CE). For *A. fumigatus*, six Burkard cyclone samples (<2–<3 CE) and one filter sample (<5 CE) were below detection; for *P. brevicompactum*, four cyclone samples (<5–<21 CE), seven Burkard cyclone samples (<6–<18 CE), and one filter sample (<18 CE) were below detection. Half the sample's reported detection limit was substituted for the PCR measurements that were below detection. The previously determined CV for PCR was 25%.

Data Analysis

Biodiversity Indices

Biodiversity is a measure of the complexity of an ecological community and includes assessments of species richness (the number of fungal groups that were observed, *S*) (Table III) and

TABLE III. Frequencies with Which Different Fungal Spore Groups were Observed by Microscopy

	Number of Samples (n_i)			
Fungal Group	**Cyclone**	**Filter**	**Burkard Slide**	**Burkard Cyclone**
Collected with all four samplers (9 fungal groups)				
Ascospores	30	30	30	30
Basidiospores	30	30	30	30
Cladosporium spp.	30	30	30	30
Aspergillus-Penicillium spp.	30	30	29	30
Myxomycetes	14	15	27	17
Ganoderma spp.	13	18	23	9
Alternaria spp.	5	8	7	8
Torula herbarum	6	4	5	5
Epicoccum spp.	2	2	2	4
Collected with three samplers (6 fungal groups)				
Oidium spp.	6	5	—	6
Chaetomium spp.	1	—	10	1
Curvularia spp.	1	—	2	2
Stemphylium spp.	2	2	—	1
Cercospora spp.	1	1	—	2
Pithomyces spp.	1	1	—	2
Collected with two samplers (3 fungal groups)				
Stachybotrys spp.	—	1	16	—
Helicosporium spp.	—	4	—	1
Drechslera/*Bipolaris* spp.	—	—	1	2
Collected with one sampler (10 fungal groups)				
Ceratosporium spp.	—	—	—	2
Nigrospora spp.	—	—	—	2
Yeast	—	—	—	1
Memnoniella sp.	—	—	1	—
Polythrincium sp.	—	—	1	—
Scopulariopsis sp.	—	1	—	—
Smuts	—	1	—	—
Triposporium sp.	—	1	—	—
Periconia sp.	1	—	—	—
Ulocladium sp.	1	—	—	—
Total observations (N)	174	184	214	185
Number of groups (S)	17	18	15	20
Other spores and cell fragments				
Cladosporium spp. conidiophore	—	6	—	2
Hyphal fragment	18	20	29	24
Unknown	7	15	—	12

evenness (the degree to which observations were distributed equally among fungal groups).[22] Indices were calculated for two types of observations, i.e., samples and concentration: n_i = the number of samples in which fungal group i was observed and the total concentration of group i, respectively, where i = 1–28 (Table III).

The disorder in the system (or degree to which fungal groups occurred in equal proportion for each sampler) was assessed with the Shannon entropy index (H'),[23,24] the most popular such index in community ecology.[22]

$$H' = -\sum p_i \ (\ln p_i)\text{, where} \quad \textbf{(1)}$$

$p_i = \frac{n_i}{N}$,

n_i = total observations (i.e., samples, Table III, or concentration) for the i^{th} fungal group, where $i = 1$ to S,

N = total observations for all fungal groups, Table III; $N = \sum n_i$.

The exponent of H' ($\exp H'$) estimates the number of equally common groups that would produce the observed H' and is a true index of diversity.[25] Minimum and maximum values for $\exp H'$ were 1 (a single group dominated) and S (all groups were represented equally). Typical $\exp H'$ values range from 4.5 to 33 and rarely exceed 90.[26]

Pielou[23] pointed out that merely stating that a population has a certain diversity value is not very informative and that a measure of evenness also should be reported. Therefore, H' was used to calculate the Shannon evenness index (E), which measures the degree to which a particular sampler reflected the maximal diversity that was possible given the observed richness of fungal groups.[22] E is a ratio that ranges from 0 (groups very uneven) to 1 (all groups represented equally).

$$E = \frac{H'}{\ln S}. \quad \textbf{(2)}$$

Similarity between fungal groups for the cyclone and comparison samplers was measured with the Morisita-Horn similarity index (C_{MH}).[27]

$$C_{MH} = 2\sum \frac{(an_i\ bn_i)}{(da + db)(aN\ bN)}, \text{where}$$
an_i = total observations for the i^{th} fungal group for sampler A,
bn_i = total observations for the i^{th} fungal group for sampler B,
$da = \sum \frac{an_i^2}{aN^2}$,
$db = \sum \frac{bn_i^2}{bN^2}$, **(3)**
aN = total observations for all fungal groups for sampler A,
bN = total observations for all fungal groups for sampler B.

C_{MH} is not influenced by sample size or richness and is not highly sensitive to the abundance of the most dominant group. Wolda[28] recommended C_{MH} as the best overall measurement of similarity. C_{MH} also is a ratio that ranges from 0 (no similarity between samplers) to 1 (perfect similarity between samplers).

Agreement Between Samplers and Analyses

Agreement between the cyclone and comparison samplers and between the two analyses was quantified through comparison of the ratio (R) vs. average (A) of paired, natural logarithm (ln), concentration measurements.[8,13,29,30] Values of R were plotted against A, the line of best agreement was determined by regression of R on A ($\hat{R} = \alpha + \beta A$), bias was estimated as the mean ratio ($\overline{R}$), and variation around $\overline{R}$ was estimated by the standard deviation (SD) of R. Upper and lower limits of agreement (LA) identified the intervals containing 95% of R. Rs and SDs could vary with concentration. Therefore, bounds were calculated not as parallel lines (i.e., $\overline{R} \pm 2$ SD) or as 95% confidence intervals (CI) wider at both extremes than in the center. Instead, the LA were determined by modeling the variability in the SDs directly as a function of the measurement level (estimated by A) using a method based on absolute residuals ($|r|$) from a fitted regression line (where $r = R - \overline{R}$). Predicted residuals ($\hat{r}$) were determined by regressing r on A, and the LA were $\hat{R} \pm t_{30(2.5\%)}\sqrt{\pi / 2\hat{r}}$, where $t_{30(2.5\%)}$ was the critical value from the Student's t-distribution for 30 samples.[29] CIs on the LA identified measurements that might be considered outliers.[30]

This comparison highlighted features such as whether the cyclone (or one analysis) over- or underestimated another (i.e., $R > 1$ or < 1, respectively); whether R varied with spore concentration (i.e., slope, $\beta \neq 0$ for R on A plots); and whether variation around $\overline{R}$ was the same across all spore concentrations or greater at one end of the distribution (i.e., upper and lower LA parallel or not, respectively). Two methods could be considered interchangeable if R clustered tightly around one with β close to zero, and if desired, one measurement could be converted to the other if any observed deviations from this pattern were consistent.

RESULTS

Analysis of variance showed no significant differences due to sampler position. For the 28 recognizable spore categories, 9 groups were collected by all four samplers in 2–30 samples, another 9 groups were seen with two or three samplers in 1–16 samples, and the remaining 10 groups were collected by one sampler only in 1–2 samples (Table III).

The four samplers agreed in their representation of the relative abundance of the major spore groups and the dominance of basidiospores, although proportionally more ascospores but fewer *Cladosporium* spp. and *Aspergillus-Penicillium* spp. were observed with the Burkard slide than the other three samplers (Figure 2). Hyphal fragments were observed in low concentrations in all but one Burkard slide sample (97%) and in more than half of the cyclone, filter, and Burkard cyclone samples (60%, 67%, and 80% of samples; 0.5%, 0.4%, 0.2%,

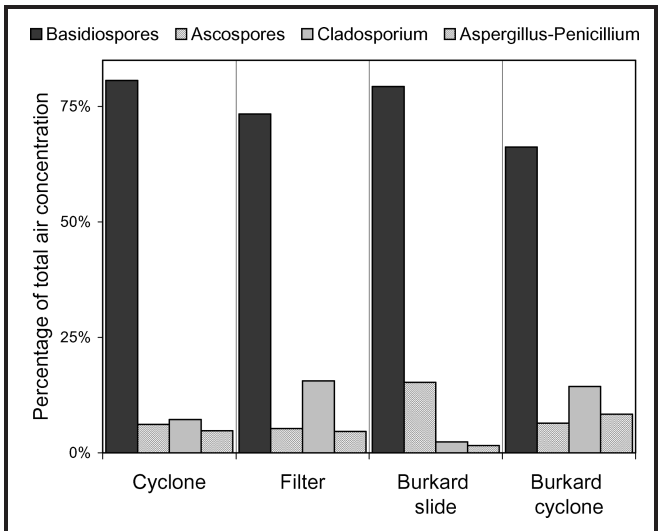

FIGURE 2. Average fractions of major spore groups (N = 30).

TABLE IV. Fungal Diversity, Evenness, and Similarity Measurements for Microscopy Analysis (N = 30)

Index	Cyclone	Filter	Burkard Slide	Burkard Cyclone
Shannon diversity, exp H'	Number of equal groups (possible range: 0–28)			
Samples	9.2	9.8	9.9	10.8
Concentration	2.1	2.4	2.0	3.1
Shannon evenness, E	Fraction of maximal diversity (possible range: 0–1)			
Samples	0.78	0.79	0.85	0.79
Concentration	0.26	0.30	0.25	0.37
Morisita-Horn similarity, C_{MH}	Fraction of maximal similarity between cyclone and comparison sampler (possible range: 0–1)			
	Samples	0.99	0.93	0.99
	Concentration	0.99	0.99	0.98
	(number of groups in common)	(13)	(11)	(15)

and 1.1% of total spore concentrations, respectively) (Table III). Unknown spores also were observed for all but the Burkard slide sampler (<0.3% of total spore concentration). *Cladosporium* spp. conidiophores (structures on which conidia are formed) were observed in six filter and two Burkard cyclone samples (<0.1% of total spore concentration). These structures were not included in total spore concentrations nor in the calculation of diversity, evenness, and similarity indices.

Culturable fractions of spores were low (<1%–8% of total spores) for the seven sample sets analyzed by both microscopy and culture but tended to be highest for the Burkard cyclone (mean: 4.8%) and lowest for the filter sampler (mean: 0.8%) (cyclone mean: 1.6%). For both microscopy and culture, the number of spore groups that were observed tended to increase with sampling time and total spore concentration (data not shown). This may reflect greater opportunity to capture spores released at different times throughout the day if sampling began earlier or ended later as well as greater fungal variety on days conducive to spore release, which resulted in higher total spore concentrations.

Fungal Diversity

The diversity indices were in the typical range of 4.5–33 (Table IV).[26] Table III shows that 15–20 groups were observed with unequal frequencies (n_i), whereas exp H' in Table IV indicates that 9–11 groups would have produced the same diversity scores had the groups been observed equally often. Calculation of diversity using fungal concentration produced much lower scores (exp $H' = 2.0$–3.1) due to the dominance of basidiospores, ascospores, and *Cladosporium* spp. (Table IV, Figure 2). Likewise, the disproportional distribution of fungal concentrations was obvious in the lower concentration than sample evenness indices ($E = 0.26$–0.37 vs. 0.78–0.85, respectively). However, the cyclone was determined to be more similar to the Burkard slide sampler if spore concentration was considered ($C_{MH} = 0.98$) rather than just presence of the individual groups ($C_{MH} = 0.93$) (Table IV).

Comparability of Air Samplers

Ratios identified how the cyclone performed relative to the other bioaerosol samplers (Table V). No bias was observed between the cyclone and filter samplers for total spores or the four major groups nor between the cyclone and Burkard slide samplers for *Cladosporium* spp. ($\overline{R}$ not significantly different from one). Plots of R vs. A revealed that agreement between samplers varied with spore concentration ($\beta \neq 0$, except for the cyclone:filter Rs for *Aspergillus-Penicillium* spp.). The variability of R either was uniform across spore concentration (e.g., Figure 3a) or greater at lower concentrations (e.g., Figure 3b), i.e., the LA were wider at the left-hand side of the distribution. Three observations exceeded the 95% CIs on the LA and, therefore, could be considered outliers but were not

TABLE V. Mean Ratios ($\overline{R}$) of Paired Cyclone and Comparison Sampler Spore Measurements (with 95% CIs; N = 30)

Comparison—Samplers	Total Spores	Basidio spores	Ascospores	*Cladosporium* spp.	*Aspergillus-Penicillium* spp.
Cyclone:Filter	**0.99** (0.81–1.17)	**1.00** (0.84–1.16)	**0.96** (0.73–1.20)	**0.95** (0.03–1.86)	**0.96** (0.57–1.34)
Cyclone:Burkard slide	0.92 (0.79–1.04)	0.91 (0.77–1.05)	0.74 (0.55–0.93)	**1.09** (0.52–1.66)	1.12 (0.62–1.63)
Cyclone:Burkard cyclone	1.33 (1.12–1.54)	1.43 (0.99–1.86)	2.31 (−1.89–6.51)	1.91 (−0.26–4.07)	1.62 (0.40–2.85)

Note: $\overline{R}$ in bold not significantly different from one.

TABLE VI. Mean Ratios ($\overline{R}$) of Paired Spore and CE Analyses (with 95% CIs; N = 30)

Comparison—Analyses	Cyclone	Filter	Burkard Cyclone
A. fumigatus CE: total *Aspergillus-Penicillium* spp. spores	0.66 (−0.07–1.39)	0.69 (−0.12–1.50)	0.35 (−0.88–1.57)
P. brevicaulis CE: total *Aspergillus-Penicillium* spp. spores	0.54 (0.06–1.03)	0.64 (0.07–1.20)	0.27 (−0.65–1.20)
C. cladosporioides CE: total *Cladosporium* spp. spores	1.26 (0.54–1.99)	1.30 (−0.06–2.65)	1.69 (−0.44–3.83)
C. herbarum CE: total *Cladosporium* spp. spores	**0.96** (0.10–1.82)	**0.99** (−0.17–2.14)	−0.10 (−3.16–2.96)

Note: $\overline{R}$ in bold not significantly different from one.

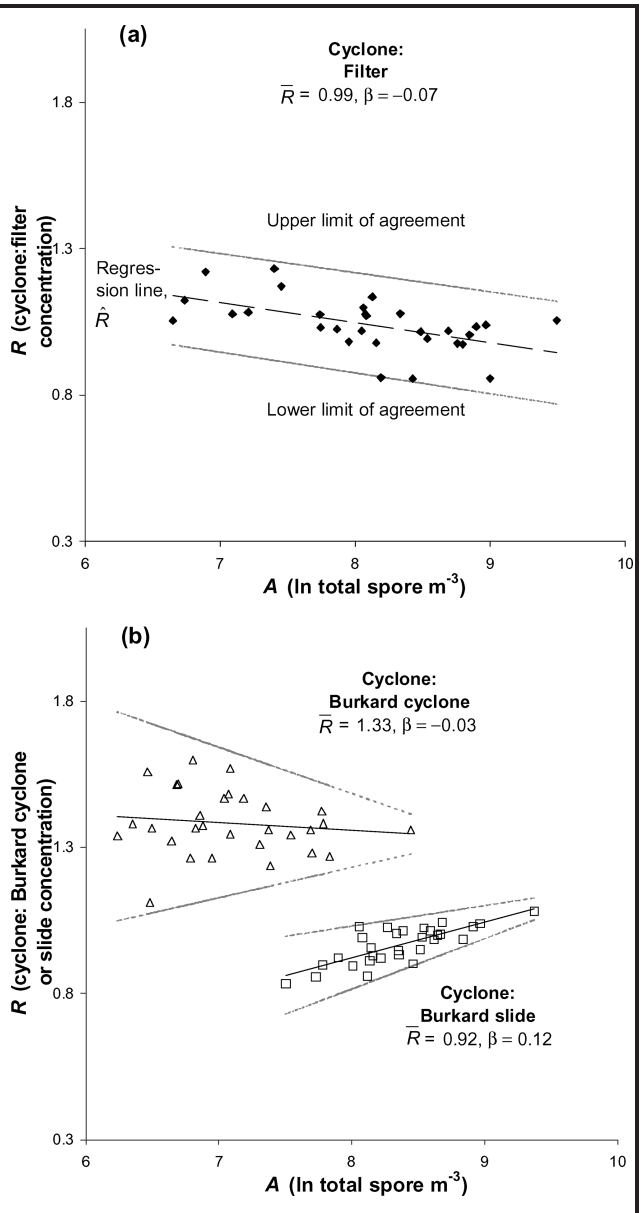

FIGURE 3. Examples of ratio (R) vs. average (A) plots illustrating the comparability of paired cyclone and comparison sampler measurements of total spore concentration (N = 30): (a) cyclone agreement with the filter sampler, and (b) cyclone agreement with the Burkard slide and cyclone samplers.

deleted from the data sets (ascospores—one cyclone:Burkard cyclone R; and *Cladosporium* spp.—one cyclone:Burkard cyclone and one cyclone:filter R; data not shown).

R and A for the cyclone:filter comparisons also were calculated for paired spore counts rather than concentrations (four major fungal groups and total spores) to remove the effect of sampling time (data not shown). $\overline{R}$ did not change greatly, but the LA for count ratios tended to be narrower and more parallel, indicating even better agreement for count than for concentration comparisons. Nevertheless, comparisons still were more variable for lower spore numbers, indicating greater uncertainty for measurements based on lower counts.

Comparability of Spore Count and PCR Analyses

R and A for paired CE and spore measurements for the cyclone, filter, and Burkard cyclone were calculated (Table VI) and plotted, as above. R was negative if a PCR measurement was <1 CE m^{-3}. Agreement between the assay methods was reasonable for *Aspergillus* spp. and *Penicillium* spp., although CE measurements were lower than total spore concentrations ($\overline{R}$ = 0.27–0.69). For the *Cladosporium* spp., CE measurements equaled or exceeded total spore measurements ($\overline{R}$ = 0.96–1.69) except for the Burkard cyclone for *C. herbarum* ($\overline{R}$ = –0.10). No observations were beyond the CIs on the LA for any of the assay comparisons, but R was correlated with A ($\beta \neq 0$).

R and A were calculated for spore:CE count data for the cyclone, filter, and Burkard cyclone to remove the effect of sampling time, as above (data not shown). Comparisons based on counts rather than concentrations again indicated better agreement, especially for the Burkard cyclone. As for the cyclone:filter comparisons, the magnitude of the differences between the analytical methods depended on the number of spores or CEs that were detected, and disagreement was greater for lower numbers.

DISCUSSION AND CONCLUSIONS

Fungal Diversity

Mycologists use biodiversity to describe fungal communities across space and time in various ecosystems,[22] and such analysis has proven informative to compare airborne fungi inside and outside office buildings in the summer and winter seasons.[31] All samplers in the cyclone evaluation identified a rich environment (S = 15–20 groups) and agreed

on the occurrence of the most common fungi (which could be explained by 9–11 equal groups) (Table IV). These values were lower but similar to what was observed outdoors at 44 U.S. office buildings using another Burkard slit impactor (S = 26 groups, summer and winter: 26 and 17 groups, respectively; overall: exp H = 17 equal groups; summer and winter: 18 and 11 equal groups, respectively).[31] However, the concentration distributions in the cyclone evaluation could be explained by just two to three equal groups due to the dominance of basidiospores, ascospores, and *Cladosporium* spp. (66%–81%, 5%–15%, and 2%–16% of total concentration, respectively) (Figure 2). For samples collected near outdoor air intakes in the office study, *Cladosporium* spp. were the most abundant group followed by ascospores, basidiospores, and *Aspergillus-Penicillium* spp. (44%, 27%, 14%, and 6% of total concentration, respectively).

The maximal diversity in the cyclone study (78%–85%, Table IV) also was lower than in the office survey (overall: 86%; summer and winter: 89% and 85%, respectively),[31] which may reflect the limited time span as well as the single season and location of the field evaluation. The fungal communities described with the cyclone and comparison samplers were nearly identical (93%–99% similarity) (Table IV).

Combining classical microscopy and culture assays with molecular techniques may help researchers understand fungal distributions in indoor and outdoor air and may provide insights into the ecology and population dynamics of fungal species.[6] Biodiversity and similarity indices could be used for comparisons other than sampler and analysis performance, e.g., to study environments before and after interventions that might alter microbial communities (such as remediation of indoor fungal contamination) as well as microbial growth on different substrata (such as wood and wallboard).

Comparability of Samplers and Analyses

A graphical procedure that is simple to perform and interpret revealed that disagreement between the cyclone and comparison samplers and between microscopy and PCR depended on the number of spores examined, with concentrations calculated from lower counts less reliable than those from higher counts.[32] The number of spores collected depended on sample volume, and although all samplers were operated for the same time period they differed greatly in airflow rate (Table I). The 4-L m^{-3} cyclone and filter samplers were the most comparable ($\overline{R}$ = 1.0) (Table V), but mean volume for these samplers was only 24% and 40% of the Burkard cyclone and slide samplers, respectively.

Airflow rate was not measured for the Burkard slide due to the inlet design and inaccessibility of the exhaust airstream. Although the sampler was calibrated prior to the study, an error in the estimation of sample volume may have contributed to the difference between this sampler and the cyclone ($\overline{R}$ = 0.7–1.1, Table V). The maximum particle size that could be collected from still air without bias due to particle inertia was much larger for the Burkard slide relative to the cyclone sampler due to its larger inlet (2 × 14 vs. 2 mm) and higher airflow rate (10 vs. 4 L min^{-1}).[33] In addition, the upward-facing slit inlet may have allowed overcollection of spores not in the original still air volume.[33]

The cut points for the personal and Burkard cyclones are similar (d_{50} = 1.5 and 1.2 μm, respectively) (Table I), but the larger cyclone consistently collected fewer spores ($\overline{R}$ = 1.3–2.3, Table V) even though its inlet and flow rate were greater (3 × 9 mm and 16 L min^{-1}, respectively). An error in the sampling rate is unlikely to explain the lower concentration measurements for this sampler because performance was similar with the Burkard cyclone's internal pump or an external air mover for which the flow rate was measured. The potential benefits of backup filters for cyclone samplers (to collect particles that may be lost due to bounce), washing of inlets and outlets (to recover particles deposited there), and coating the MCT surfaces (to retain particles) have been discussed previously.[8]

The culturable fractions of spores ranged from <1%–8%, much lower than Adhikari et al.[12] and Tsai et al.[31] observed outdoors (30%–>100% and 25%, respectively). However, the other studies used different samplers to make the two measurements rather than conducting both analyses on the same spore suspensions as done here for the cyclone, filter, and Burkard cyclone samples. The others also observed more *Cladosporium* spp. but fewer ascospores and basidiospores, which dominated in the California study and may be difficult to grow on laboratory culture medium.[34] Some spores may not have survived the very long sampling periods in this field study (7–19 hr), especially on filters. In addition, the delay from the time of collection to analysis may have reduced spore viability. The low culturability of ambient spores illustrates the value of microscopy and molecular methods for measurement of fungal air concentrations.

Unfortunately, PCR was not available for the most abundant fungal groups, i.e., basidiospores and ascospores. R for *C. cladosporioides* and *C. herbarum* CE:spore concentrations equaled or exceeded one (except for the Burkard cyclone sampler) (Table VI), suggesting that these two (of three *Cladosporium* spp. identified by culture or PCR) accounted for the majority of total *Cladosporium* spp. spores. CE measurements also may have exceeded spore counts because the PCR analysis detected DNA in unidentifiable spores and cell fragments, e.g., conidiophores, hyphal fragments, and other cell debris.[35] Fragments of hyphae detached from spores are impossible to identify taxonomically using a light microscope.[36] CE:spore ratios were below one for *A. fumigatus* and *P. brevicompactum* relative to total *Aspergillus-Penicillium* spp., but these were only two of seven species in this group (four *Aspergillus* spp. and three *Penicillium* spp.). The lower concentrations of these genera relative to *Cladosporium* spp. also may have resulted from less efficient collection of the smaller spores, greater difficulty observing the spores by microscopy, as well as the greater uncertainty in these measurements because they were based on counting fewer spores or CEs (Table II).

No sampler collected all 28 of the identifiable spore types, all 16 of the cultured species, nor all 14 PCR-positive species. However, diversity and evenness indices for the four samplers agreed and were similar to what has been observed nationally. Therefore, the cyclone performed as well as other aerobiological samplers to describe the fungi in outdoor air. Consistent trends in plots of paired *R* vs. *A* indicated excellent agreement between the cyclone and filter samplers, good agreement with the Burkard slide, and reasonable agreement with the Burkard cyclone as well as between microscopy and PCR. Therefore, reference sampler measurements could be predicted accurately from cyclone data and measurements for the two analyses approximated from each other. The cyclone was the most compact of the four samplers tested and was the most convenient to prepare for sample collection and analysis, considerations important for field studies. Therefore, the personal cyclone may be considered for collection of indoor and outdoor airborne spores and for analysis by traditional and advance assay methods.

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REFERENCES

1. **Chew, G.L., J. Wilson, F.A. Rabito, et al.:** Mold and endotoxin levels in the aftermath of Hurricane Katrina: A pilot project of homes in New Orleans undergoing renovation. *Environ. Health Perspect. 114*:1883–1889 (2006).
2. **MacNeil, L., T. Kauri, and W. Robertson:** Molecular techniques and their potential application in monitoring the microbiological quality of indoor air. *Can. J. Microbiol. 41*:657–665 (1995).
3. **McCartney, H.A., S.J. Foster, B.A. Fraaije, and E. Ward:** Molecular diagnostics for fungal plant pathogens. *Pest. Manag. Sci. 59*:129–142 (2003).
4. **Williams, R.H., E. Ward, and H.A. McCartney:** Methods for integrated air sampling and DNA analysis for detection of airborne fungal spores. *Appl. Environ. Microbiol. 67*:2453–2459 (2001).
5. **Zhou, G., W.-Z. Whong, and B. Chen:** Development of a fungus-specific PCR assay for detecting low-level fungi in an indoor environment. *Mol. Cell. Probes 14*:339–348 (2000).
6. **Schmit, J.P., and D.J. Lodge:** Classical methods and modern analysis for studying fungal diversity. In *Fungal Community: Its Organization and Role in the Ecosystem*. Boca Raton, Fla.: Taylor & Francis, 2005. pp. 193–214.
7. **Chen, B.T., G.A. Feather, A. Maynard, and C.Y. Rao:** Development of a personal sampler for collecting fungal spores. *Aerosol Sci. Technol. 38*:926–937 (2004).
8. **Macher, J.M., B.T. Chen, and C.Y. Rao:** Chamber evaluation of a personal, bioaerosol cyclone sampler. *J. Occup. Environ. Hyg.* 5:702–712.
9. **Hung, L.L., J.D. Miller, and H.K. Dillon (ed.):** Total fungi and other assessment methods. In *Field Guide for the Determination of Biological Contaminants in Environmental Samples*, 2nd ed. Fairfax, Va.: AIHA, 2005. pp. 141–208.
10. **Airaudi, D., and V.F. Marchisio:** Fungal biodiversity in the air of Turin. *Mycopathologia 136*:95–102 (1996).
11. **Rainer, J., U. Peintner, and R. Poder:** Biodiversity and concentration of airborne fungi in a hospital environment. *Mycopathologia 149*:87-97 (2001).
12. **Adhikari, A., M.M. Sen, S. Gupta-Bhattacharya, and S. Chanda:** Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: A 2-year study at five outdoor sampling stations. *Sci. Total Environ. 326*:123–141 (2004).
13. **Bland, J.M., and D.G. Altman:** Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet 1(8476)*:307–310 (1986).
14. **Macher, J.M., and H.A. Burge:** Sampling biological aerosols. In *Air Sampling Instruments for Evaluation of Atmospheric Contaminants*. 9th ed. Cincinnati, Ohio: ACGIH®, 2001. pp. 661–701.
15. **Hinds, W.C.:** Filtration. In *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles*, 2nd ed. New York: John Wiley & Sons, Inc., 1999. pp. 182–205.
16. **Hinds, W.C.:** Filtration. In *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles*, 2nd ed. New York: John Wiley & Sons, Inc., 1999. pp. 111–140.
17. **British Aerobiological Federation:** *Airborne Pollens and Spores: A Guide to Trapping and Counting*. Aylesford, Kent, UK: Kimberly Clark Ltd., 1995.
18. **Brinkman, N.E., R.A. Haugland, L.J. Wymer, M. Byappanahalli, R.L. Whitman, and S.J. Vesper:** Evaluation of a rapid, quantitative real-time PCR method for enumeration of pathogenic *Candida* cells in water. *Appl. Environ. Microbiol. 69*:1775–1782 (2003).
19. **Meklin, T., R.A. Haugland, T. Reponen, et al.:** Quantitative PCR analysis of house dust can reveal abnormal mold conditions. *J. Environ. Monit. 6*:615–620 (2004).
20. **Haugland, R.A., N.E. Brinkman, and S.J. Vesper:** Evaluation of rapid DNA extraction methods for the quantitative detection of fungal cells using real time PCR analysis. *J. Microbiol. Methods 50*:319–323 (2002).
21. **Samson, R.A., E.S. Hoekstra, C. Frisvad, and O. Filtenborg (eds.):** Identification of the common food- and airborne fungi. In *Introduction to Food- and Airborne Fungi*, 6th ed. Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures, 2000. pp. 1–282.
22. **Zak, J.C., and M.R. Willig:** Fungal biodiversity patterns. In *Biodiversity of Fungi: Inventory and Monitoring Methods*, G.M. Mueller, G.F. Bills, and M.S. Foster (eds.). New York: Elsevier Academic Press, 2004. pp. 59–75.
23. **Pielou, E.C.:** The measurement of diversity in different types of biological collections. *J. Theor. Biol. 13*:131–144 (1966).
24. **Shannon, C.E., and W. Weaver:** *The Mathematical Theory of Communication*. Urbana, IL: University of Illinois Press, 1969.
25. **Jost, L.:** Entropy and diversity. *Oikos 113*:363–375 (2006).
26. **Margalef, R.:** Homage to Evelyn Hutchinson, or why there is an upper limit to diversity. In *Transactions: Growth by Intussesception: Ecological Essays in Honor of G. Evelyn Hutchinson*, Vol. 44. New Haven, Conn.: Connecticut Academy of Arts and Sciences, 1972. pp. 211–235.
27. **Horn, H.:** Measurement of "overlap" in comparative ecological studies. *Am. Naturalist 100*:419–424 (1966).
28. **Wolda, H.:** Similarity indices, sample size and diversity. *Oecologia (Berlin) 50*:296–302 (1981).
29. **Bland, J.M., and D.G. Altman:** Measuring agreement in method comparison studies. *Stat. Methods Med. Res. 8*:135–160 (1999).
30. **Bland, J.M., and D.G. Altman:** Applying the right statistics: Analyses of measurement studies. *Ultrasound Obstet. Gynecol. 22*:85–93 (2003).

31. **Tsai, F.C., and J.M. Macher:** Concentrations of airborne culturable fungi and fungal spores in large U.S. office buildings from the BASE study. *Atmos. Environ. 41*:5181–5191 (2007).
32. **Wu, Z., G. Blomquist, S.-O. Westermark, and X.-R. Wang:** Application of PCR and probe hybridization techniques in detection of airborne fungal spores in environmental samples. *J. Environ. Monit. 4*:673–678 (2002).
33. **Hinds, W.C.:** Sampling and measurement of concentration. In *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles*, 2nd ed. New York: John Wiley & Sons, Inc., 1999. pp. 206–232.
34. **Lodge, D.J., J.F. Ammirati, T.E. O'Dell, and G.M. Mueller:** Terrestrial and lignicolous macrofungi. In *Biodiversity of Fungi: Inventory and Monitoring Methods*, G.M. Mueller, G.F. Bills, and M.S. Foster (eds.). New York: Elsevier Academic Press, 2004. pp. 127–172.
35. **Zeng, Q.Y., S.O. Westermark, Å. Rasmuson-Lestander, and X.R. Wang:** Detection and quantification of *Cladosporium* in aerosols by real-time PCR. *J. Environ. Monit. 8*:153–160 (2006).
36. **Hjelmroos-Koski, M.K., J.M. Macher, S.K. Hammond, and I.B. Tager:** Considerations in the grouping of plant and fungal taxa for an epidemiologic study. *Grana 45*:261–287 (2006).