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Comparison of quantitative airborne fungi measurements by active and passive sampling methods

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ABSTRACT

The present study compared the airborne fungi collection performance of a two-stage cyclone sampler (active method) to the performance of the Personal Aeroallergen Sampler (passive method) using quantitative polymerase chain reaction (qPCR) assays. Indoor air concentrations of the common fungal species *Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, and *Penicillium chrysogenum* were considered. Good correlations between the two sampling methods for the fungi *A. alternata*, *C. cladosporioides*, and *E. nigrum* were observed and the mean effective passive sampling rates ($\pm$std. dev.) for these species were 0.032 ($\pm$0.006), 0.058 ($\pm$0.006), and 0.066 ($\pm$0.044) l min^{-1}, respectively. Gravitational settling was the dominant collection mechanism for *A. alternata* and *E. nigrum*. The root mean square precisions for the passive sampler measurements were also comparable to those of the active sampler (49–73% and 50–102%, respectively). The passive sampler did not allow for the collection of *P. chrysogenum*, likely due to the insufficient gravitational settling velocity of the fungal particle with its aerodynamic diameter of less than 5 μm. The passive sampler, in conjunction with growth-independent qPCR detection methodologies, can be utilized in future exposure assessment studies to deepen our understanding of how individuals are affected by inhalation of airborne fungal pathogens and allergens, especially for those with an aerodynamic diameter greater than 5 μm.

1. Introduction

Type I hypersensitivity reactions including allergic asthma and allergic rhinitis are a major cause of illness and disability in the developed world, and exposure to airborne allergens is a strong risk factor for asthma (Ring et al., 2001). Fungi are ubiquitous in indoor and outdoor environments and many fungi are known to cause allergic reactions and exacerbate asthma attacks (Mudarri & Fisk, 2007). Additionally, invasive fungal infections such as aspergillosis and candidiasis are caused by commonly occurring environmental fungi and are highly fatal for immunocompromised patients (Lin et al., 2001). To clarify human health impacts caused by environmental fungi and to develop preventative measures, accurate and systematic exposure assessments are needed.

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Accurately assessing personal exposure to airborne fungi must include aerosol monitoring in the vicinity of the human subject. Although pump-driven active samplers have been widely used to assess personal exposures (Adhikari et al., 2004; Su et al., 2001), they are occasionally impractical because of their noise, weight, size, and limited battery lifetime. In response, we have recently developed the Personal Aeroallergen Sampler (PAAS), a passive sampler for airborne coarse particles to collect allergenic materials by gravitational settling (Yamamoto et al., 2006). To date, several types of passive samplers for airborne particles have been reported (Brown et al., 1995; Vinzents, 1996; Wagner & Leith, 2001). Unlike pump-driven active samplers, they are light, small, and silent since they do not require a pump or battery. The PAAS has previously demonstrated its applicability to collect airborne major allergens of the large ($\sim$30 μm diameter) Japanese cedar and cypress pollens in indoor and outdoor settings (Yamamoto et al., 2007, 2010).

The goal of this study was to test the applicability of the PAAS for measuring airborne fungi, which typically have a size range of 0.5–30 μm (Hinds, 1999) and are generally smaller than cedar and cypress pollen. We conducted sampling and quantitative PCR (qPCR)-based measurements in an indoor setting to compare the sampling performance of the passive sampler with that of a reference two-stage cyclone active sampler (Chen et al., 2004; Lindsley et al., 2006). Effective passive sampling rates were determined for each species considered, and the precision of the active and passive sampler-based measurements was compared. Limitations on the application of the passive sampler method are presented.

2. Experimental

2.1. Air sampling

Sampling campaigns were conducted by co-locating the two-stage cyclone sampler (Model BC 221; National Institute for Occupational Safety and Health, WV, USA; hereafter called active sampler) and the Personal Aeroallergen Sampler (PAAS) (Sibata Scientific Technology Ltd., Tokyo, Japan; hereafter called passive sampler) on a window frame of a residential building in New Haven, Connecticut, USA from September to December 2009. Overall, 25 co-located active and passive air sampling experiments that ranged in time from 3 h to 35 days were performed at the same location on different days, in which duplicated active and passive ($n=9$ each) measurements were included. Active and passive samplers were run for the exact same sampling durations. For the duplicated measurements, two same samplers (i.e., active or passive) were co-located for the exact same time durations. Sampling times varied to produce a range of fungal particle quantities collected by the passive sampler and to ensure that times were adequate to collect detectable concentrations.

The active sampler consisted of two sterile microcentrifuge tubes (1.5 ml; conical bottom; catalog number 02-681-373; Thermo Fisher Scientific Inc., MA, USA) attached to a sampler head that provided an air flow rate of 2 l min^{-1}. The sampler allowed 50% cut-off sizes at 2.6 and 1.6 μm aerodynamic diameters (d_a) for the first and second stage cyclones, respectively. The aspiration efficiency of the reference active sampler was $>98\%$ for particles with aerodynamic diameters of 3.1 μm or less, but smaller for larger particles, i.e., 89% for 6.2 μm particles (Lindsley et al., 2006). The active sampler was connected to a portable vacuum pump (MP-Σ500; Sibata Scientific Technology Ltd., Tokyo, Japan). For the passive sampler, a sterile gelatin filter (25 mm filter diameter; SKC Inc., PA, USA) was loaded into the unit for the gravitational collection of airborne fungal particles.

2.2. Fungal concentration measurement by qPCR

2.2.1. Preparation of the standard fungal DNA

These experiments considered four medically-important allergenic fungi including *Alternaria alternata* (PEM 01043; Prestige EnviroMicrobiology Inc., NJ, USA), *Cladosporium cladosporioides* (ATCC 16022), *Epicoccum nigrum* (TU BL-3; The University of Tulsa) and *Penicillium chrysogenum* (CAES PC-1; The Connecticut Agricultural Experiment Station, CT, USA). Fungi were grown on malt extract agar (Difco Laboratories, MI, USA) in the dark at 23 °C for 3 weeks. To prepare standards for qPCR, spores of *C. cladosporioides* and *P. chrysogenum* were generated by growth on malt extract agar. The spores were harvested by cotton swabs and were suspended in 10 ml of 70% ethanol with deionized water. The suspensions were placed into 1.5 ml microcentrifuge tubes and centrifuged at 10,000*g* for 3 min to pellet the spores, and the ethanol supernatant was removed. To generate the standard curves based on cell counts, the numbers of spores in the pellets were enumerated by direct microscopy (Hospodsky et al., 2010). Unlike fungal species forming unicellular amerospores such as *C. cladosporioides* and *P. chrysogenum*, accurate cell enumeration is difficult for *A. alternata* and *E. nigrum* which produce multicellular dictyospores. Therefore, tissues of a 4 cm^2 area of the colonies were collectively isolated and used to prepare the DNA standards for these two fungi, and the original numbers of fungal cells were back-calculated based on the recovered DNA quantities and genome sizes of these fungal species. Here we used the theoretical mass concentration of DNA per cell for each test organism estimated by the following: DNA mass in one cell [pg]=genome size [bp]/(0.978×10^9 [bp/pg]) (Dolezel et al., 2003). The recovered DNA was quantitated by PicoGreen (dsDNA Reagent and Kits; Invitrogen, CA, USA), and the genome size of *E. nigrum* was assumed to be 30.0 Mbp (same as *A. alternata* (Masunaka et al., 2005)) since no data are currently available. We assumed 10% of the DNA extraction efficiencies for these two fungal species as a typically observed value for *Aspergillus fumigatus* spores by our DNA extraction protocol (Hospodsky et al., 2010). The quantities of 18S rRNA gene copies are known to be highly variable by fungal strain (Herrera et al., 2009), therefore qPCR standards were based on the total numbers of spores harvested from the above-mentioned fungal strains rather than gene copies.

Table 1
Primers and probes used for the qPCR assays.

Assay name[a]	Fungal species	Primer (probe) name	Sequence 5′-3′
Aaltr	*Alternaria alternata*	AaltrF1	GGCGGGCTGGAACCTC
		AltrR1-1	GCAATTACAAAAGGTTTATGTTTGTCGTA
		AaltrP1	TTACAGCCTTGCTGAATTATTCACCCTTGTCTTT
Cclad2	*Cladosporium cladosporioides*, svar. 2	Cclad2F1	TACAAGTGACCCCGGCTACG
		CcladR1	CCCCGGAGGCAACAGAG
		CcladP1	CCGGGATGTTCATAACCCTTTGTTGTCC
Enigr	*Epicoccum nigrum*	EnigrF1	TTGTAGACTTCGGTCTGCTACCTCTT
		EnigrR1	TGCAACTGCAAAGGGTTTGAAT
		EnigrP1	CATGTCTTTTGAGTACCTTCGTTTCCTCGGC
PenGrp3	*Penicillium chrysogenum/griseofulvum/glandicola/ coprophilum/expansum* and *Eupenicillium*	PchryF1	CGGGCCCGCCTTAAC
		PchryR1-1	GAAAGTTTTAAATAATTTATATTTTCACTCAGAGTA
		PenP2	CGCGCCCGCCGAAGACA

[a] Primers and probes reported by Haugland and Vesper (2002) were used for the qPCR assays.

2.2.2. DNA extraction

DNA extraction was performed for pure culture standards as well as the active and passive sampler media using the Mobio PowerSoil DNA Isolation Kit (Mobio Laboratories Inc., CA, USA). For the passive sampler, one half of each gelatin filter after sampling was transferred to a 2 ml microcentrifuge tube and Mobio power beads (1.0 g) and lysis solution (750 ml) supplemented with 0.1 mm diameter glass beads (300 mg) and 0.5 mm diameter glass beads (100 mg) were added. Here addition of the glass beads was necessary to achieve sufficient DNA extraction. For the active sampler and for qPCR standards, the same amounts of Mobio power beads and lysis solution as described above were directly added to the 1.5 ml sampling tubes containing the environmental sample or a known amount of standard fungi. Cell walls were lysed by bead beating for 5 min at 3450 rpm (Model 607; BioSpec Products Inc., OK, USA). After bead beating, DNA was purified in accordance with the manufacturer's protocols and eluted into 50 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH=8.0).

2.2.3. Real-time qPCR

The TaqMan method was used for species-specific fungal detection and employed the Aaltr, Cclad2, Enigr, and PenGrp3 qPCR assays reported by Haugland and Vesper (2002). Selectivity of each qPCR assay is listed in Table 1. A 25 μl reaction mixture containing the template DNA (1 μl sample extract), 1 × TaqMan Universal PCR Master Mix (Applied Biosystems, CA, USA), 1 μM of each primer, and 0.08 μM of the probe was used. Sequences of primers and probes are summarized in Table 1. A real-time PCR system (ABI 7500 Fast Real-time PCR System; Applied Biosystems, CA, USA) was used with the following cycle condition: 50 °C for 2 min, 95 °C for 15 min of initial denaturation and 45 cycles of 95 °C for 15 s of dissociation and 60 °C for 1 min of annealing and extension. Cycle thresholds were calculated by using the auto function in ABI 7500. All qPCR measurements were performed in triplicate. To test for PCR inhibition, seven randomly selected extracts from indoor air samples collected in this study were added to subsets of diluted *E. nigrum* DNA standard and standard curves were produced. No significant inhibition was observed.

2.3. Precision of the duplicated measurements

The two major sources of variations in these measurements include variations derived from air sampling and from the qPCR method. The overall measurement precisions obtained in this study were compared with qPCR precisions previously reported for fungal cells (Hospodsky et al., 2010). To determine the overall measurement precision of both sampling methods, the root mean square (RMS) precision (equivalent to the averaged coefficient of variation across all paired samples) for n pairs of the duplicated active or passive measurements, i.e., (x_{11}, x_{12}), (x_{21}, x_{22}), $(x_{31}, x_{32})\sim(x_{n1}, x_{n2})$, was calculated according to the following equation given by Hyslop and White (2009):

$$\text{RMS precision} = \sqrt{\frac{1}{n}\sum_{i}^{n}\left[\frac{(x_{i1}-x_{i2})/\sqrt{2}}{m_i}\right]^2} \quad (1)$$

where m_i is the arithmetic mean (average) of ith paired data. As shown in Eq. (1), the RMS precision is a root mean square of multiple coefficient of variation (COV) values obtained by a series of duplicated experiments. The RMS precisions were calculated for the duplicated active ($n=9$) or passive ($n=9$) sampling methods as well as both of the methods combined ($n=18$).

3. Model

3.1. Calculation of the measured deposition velocities

The measured deposition velocities as well as the theoretical gravitational settling and thermal velocities of airborne fungi were estimated and compared in order to determine if the passive sampler performance could be mostly attributed to gravitational particle deposition. A relationship for calculating fungal deposition velocities based on sampler measurement was developed by recognizing that the sampling rate of the passive sampler (F, ml min^{-1}) is the product of the effective particle deposition area of the passive sampler (A=3.8 cm^2) and the deposition velocities of airborne fungi ($V_{d,\ measured}$, cm min^{-1})

$$F = AV_{d,\ measured} \tag{2}$$

The sampling rate, F, of the passive sampler can be obtained by the ratio of the particle numbers collected by the co-located passive and active samplers and is equal to the product of the active sampler flow rate (Q=2 l min^{-1}) and the ratio of the numbers of fungal cells collected by the passive (N_p) and active samplers (N_a)

$$\frac{N_p}{N_a} = \frac{F}{Q} \tag{3}$$

3.2. Calculation of the gravitational settling velocities

The gravitational settling velocities $V_{d,\ gravity}$ (cm min^{-1}) estimates were based on Stokes' gravitational settling under the assumptions of physical sizes and shapes of fungal spores listed in Table 2 and the relationship given by Hinds (1999) and presented in the following equation:

$$V_{d,\ gravity} = \frac{\rho d_e^2 g K}{18\eta} \tag{4}$$

where ρ is the density of fungal spores (assumed to be 1.0 g cm^{-3}(Gregory, 1973)), d_e is the equivalent volume diameter, g is the acceleration of gravity (=980 cm s^{-2}), η is the viscosity of air (=1.8 × 10^{-5} Pa s), and K is the shape resistant factor. The equivalent volume diameters were calculated under the assumptions of the physical sizes and shapes of fungal spores specified in Table 2. The dimensionless dynamic shape factor was calculated by the following empirical relationship according to Johnson et al., 1987):

$$K = 0.246 + 0.531(\Psi) + 0.258(d_e/d_n) - 0.036(d_{max}/d_n) \tag{5}$$

where Ψ is the surface sphericity, d_n is the projected area diameter, and d_{max} is the maximum dimension along a principal axis. As spheres and spheroids are orthotropic, the values of K were averaged over all three axial orientations. Although fungal hyphae may represent certain proportions of bioaerosol (Wittmaack et al., 2005; Green et al., 2006), our model considered only for fungal spores because few data are available on physical properties of fungal hyphae.

3.3. Calculation of the mean thermal velocities

As the sizes of many fungal cells are in the transitional region between diffusion and gravitational settling regimes, diffusion may also affect the mechanisms associated with passive sampling. The thermal velocity is the particle velocity caused by impaction of air molecules, and the average velocity in all directions is zero. Although thermal velocity is not equal to the deposition velocity to the horizontal surface of the passive sampler since it is randomly oriented (i.e., not necessarily downward), comparison of the settling velocities with the thermal velocities can provide insight into how diffusion might affect the passive sampling mechanisms of airborne fungi. Mean thermal velocities, $\overline{u}_p$ (cm min^{-1}), were estimated for each fungal species considered (Zhang, 2005):

$$\overline{u}_p = \left(\frac{8kT}{\pi m_p}\right)^{1/2} \tag{6}$$

Table 2
Physical characteristics of fungal spores.

Fungal species	Physical size ($L \times W$, μm)	Shape	Shape resistant factor, K[a]
Alternaria alternata	18–83 × 7–18[b]	Spheroidal	0.81–0.91
Cladosporium cladosporioides	3–11 × 2–5[b]	Spheroidal	0.93–0.97
Epicoccum nigrum	15–25[b]	Spherical	1
Penicillium chrysogenum	2.5–3.5[c]	Spherical	1

[a] Orientation-averaged shape resistant factor calculated by Eq. (5) given by Johnson et al. (1987).
[b] Cole and Samson (1984).
[c] Rydjord et al. (2007).

where k is Boltzmann's constant ($=1.38\times10^{-23}$ J K^{-1}), T is the temperature ($=300$ K), and m_p is the mass for each fungal particle under the assumptions of the physical properties of fungal cells listed in Table 2.

4. Results and discussion

4.1. Relationships of airborne fungi amounts collected by the active and passive samplers

Fig. 1 illustrates the relationships between the numbers of fungal cells collected by the active and passive samplers. The data shown are mean values of triplicate qPCR measurements. Linear regression lines drawn from the origins were calculated by assuming 1.0 cell for non-detected samples. Good correlations were found for the qPCR primer-defined groups Aaltr, Cclad2 and Enigr ($r^2=0.4795$, 0.6858, and 0.7595, respectively). For the PenGrp3, few spores were detected by the passive sampler and no correlation with the active sampler quantities was found. The ratios of collected spores by the passive sampler to collected spores by the active sampler, i.e., N_p/N_a, varied for each species and were 1:64, 1:30, and 1:35 for Aaltr, Enigr, and Cclad2, respectively. Fig. 2 shows the particle size fractions of airborne fungal cells collected by the two-stage cyclone sampler. The majority of airborne fungi ($>90\%$) collected impacted into the first tube ($d_a>2.7$ μm). This was particularly true for *A. alternata* and *E. nigrum*, where $>99.9\%$ were collected in the first tube. *A. alternata* and *E. nigrum* form dictyospores larger than 10 μm while *C. cladosporioides* and *P. chrysogenum* form amerospores smaller than 10 μm in diameter (Table 2).

4.2. Measurement precision

Table 3 summarizes the RMS precisions of the qPCR measurements of airborne fungi collected by the duplicated active or passive sampling methods. The RMS precisions ranged from 41% to 102%, excluding the data of PenGrp3 by the passive method in which no sample was amplified by qPCR. The RMS precisions for the active and passive methods were 50–102% and 49–73%, respectively, excluding the data of PenGrp3 by the passive method. The precisions of the active and passive methods were not significantly different for Aaltr and Enigr ($p>0.05$), but significantly different for Cclad2 ($p<0.05$). The precision in this case can be divided into variation associated with the reproducibility of the qPCR measurements and variation associated with air sampling. Hospodsky et al. (2010) indicated that COVs of 28–79% were observed for the qPCR measurements of bacteria and fungi that were artificially spiked onto air sampling filters and that the majority of the variation was due to qPCR instrument repeatability. Given the similar precision level estimated here, the measurement variation was likely due to the qPCR

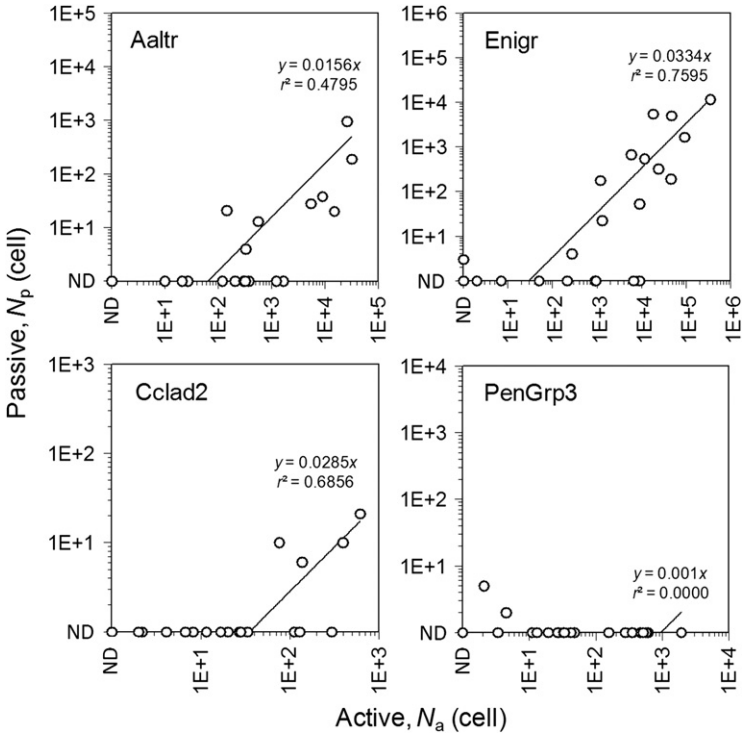

Fig. 1. Relationships between the numbers of fungal cells collected by the active and passive samplers and quantified using qPCR. 25 co-located active and passive air sampling experiments for durations ranging from 3 h to 35 days were performed at the same location on different day. Solid line represents a linear regression.

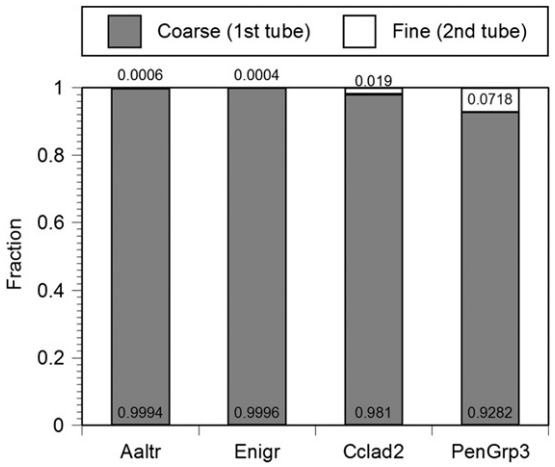

Fig. 2. Particle size distributions of airborne fungal cells collected by the two-stage cyclone sampler. Fine and coarse particles represent 1.6–2.6 and >2.6 μm of aerodynamic diameters, respectively.

Table 3
Root mean square (RMS) precisions of the qPCR measurements of airborne fungi collected by the duplicated active or passive sampling methods.

Method	Assay name	RMS precision (%)
Passive ($n=9$)	Aaltr	60
	Cclad2	49
	Enigr	73
	PenGrp3	0[a]
Active ($n=9$)	Aaltr	50
	Cclad2	102
	Enigr	67
	PenGrp3	58
Passive+active ($n=18$)	Aaltr	55
	Cclad2	80
	Enigr	70
	PenGrp3	41

[a] No sample was amplified by qPCR.

Table 4
Measured velocities, and theoretical gravitational settling and mean thermal velocities (cm s^{-1}) of airborne fungi.

Fungal species	$V_{d,\ measured}$	$V_{d,\ gravity}$ [a]	$\bar{u}_p$ [b]
Alternaria alternata	0.14 ($\pm$0.03)[c]	0.25–2.21	0.003–0.015
Cladosporium cladosporioides	0.25 ($\pm$0.03)[c]	0.02–0.12	0.03–0.13
Epicoccum nigrum	0.29 ($\pm$0.19)[c]	0.68–1.89	0.0036–0.0077
Penicillium chrysogenum	ND[d]	0.02–0.04	0.07–0.11

[a] Calculated based on Stokes' law given by Eq. (4).
[b] Calculated by Eq. (6).
[c] Parentheses indicate standard errors of mean estimates of $V_{d,\ measured}$.
[d] Not determined.

reproducibility and sample processing, which is large when compared to the variation associated with either passive or active air sampling process.

4.3. Deposition velocities of airborne fungi

Table 4 summarizes the measured velocities, and theoretical gravitational settling and mean thermal velocities of airborne fungi. The slopes in Fig. 1 were used as the values of N_p/N_a in Eq. (3) to obtain the measured deposition velocities. Standard errors of the mean estimates of $V_{d,\ measured}$ were also characterized based on the standard errors of the slopes.

Here we assumed an active sampler aspiration efficiency of 100% in order to calculate the measured deposition velocities. However, there is no data on the sampling efficiencies for particles larger than 10 μm. It is important to note that the reported measured velocities may be overestimated due to this assumption (Lindsley et al., 2006).

Nevertheless, the result showed good agreements between the measured and theoretical gravitational settling velocities. The measured velocities were in the same order of magnitude as (or slightly smaller than) the theoretical gravitational settling velocities, with the exception of *C. cladosporioides*, for which the measured velocities were slightly larger than the theoretical gravitational settling velocities. The smaller deposition velocities calculated for the passive sampler were expected due to the effects of the sampler's geometry. Since the passive sampler collects particles on the substrate loaded in the hollow of the substrate holder and covered by a protective mesh and tightened by a stainless filter holder cap (Yamamoto et al., 2006), the underlying geometry partially prevents the particle deposition flux to the collection substrate. Indeed, Yamamoto et al. (2006) reported that particles with projected area diameters of 10–100 μm were underestimated by 43–73% due to the shading effect associated with the sampler's geometry.

As sizes of fungal cells are partially in the transitional region between diffusion and gravitational settling regimes, diffusion might also affect the passive sampling mechanisms. To assess the significance of diffusion, mean thermal velocities for each fungal species were calculated and are presented in Table 4. The thermal velocities of *A. alternata* and *E. nigrum* were approximately 2–3 orders of magnitude smaller than the corresponding settling velocities (Table 4), suggesting diffusion was insignificant for these species. Meanwhile, the thermal velocities of *C. cladosporioides* and *P. chrysogenum* were comparable to the corresponding settling velocities. Thus, although diffusion was likely negligible for species forming multicellular spores larger than 10 μm such as *A. alternata* and *E. nigrum*, it may contribute to the collection of fungal species producing unicellular spores smaller than 10 μm such as *C. cladosporioides* and *P. chrysogenum*. Indeed, Table 4 shows *C. cladosporioides* had higher measured deposition velocities than theoretical gravitational settling velocities, suggesting a potential contribution of diffusion against the overall passive sampling mechanisms.

Our model calculation considered only for fungal spores. While certain proportions of airborne fungi are known to be in the hyphal form (Wittmaack et al., 2005; Green et al., 2006) and fungal fragments (Reponen et al., 2007), which are too small to be collected by the passive sampling, we found generally good agreement between the measured and theoretical gravitational settling velocities.

4.4. Sampling rates of airborne fungi by the passive sampler

Table 5 summarizes the sampling rates of airborne fungi by the passive sampler. Aerosol concentrations of airborne fungi, *C*, can be characterized by using these sampling rates, the number of spores sampled, and the sampling duration. The sampling duration by the passive sampler can also be optimized by considering the sampling rates of target fungi and the concentrations in given environments. Fig. 3 illustrates times required to collect 100 cells by the passive sampler when calculated based on the sampling rates presented in Table 5. Here we assumed 100 fungal cells as a minimum detectable amount based on previous method detection level estimations for fungal aerosols by qPCR (Hospodsky et al., 2010). The passive sampler is particularly suitable for occupational settings such as agricultural environments since concentrations reach 10^6 spores m^{-3} and potentially up to 10^9 spores m^{-3} or more (Lacey & Dutkiewicz, 1994), but may not be well suited for environments where levels or agents are below 10^3 m^{-3}, particularly if the chosen spore has an aerodynamic diameter below 5 μm.

Finally, in outdoor environments, particle collection efficiencies by passive sampling could be influenced by wind velocity as inertial deposition caused by atmospheric turbulence could also contribute to sampling collection (Kim et al., 2000). Indeed, Yamamoto et al. (2006) reported the particle collection efficiencies indoors were different from those outdoors owing to difference in wind velocity. Accurate measurements for the passive sampler are possible in indoor environmental settings as indoor air velocities are typically at a level of 10 cm s^{-1} in occupied and unoccupied dwellings (Matthews et al., 1989) and inertial deposition due to air disturbance is insignificant at this level. To implement passive sampling outdoors, care must be taken to choose mild wind locations and/or using a shelter to protect the sampler from high wind exposures (Ott & Peters, 2008).

Table 5
Effective sampling rates of airborne fungi by the passive sampler.

Fungal species	Sampling rate, F (l min^{-1})
Alternaria alternata	0.032 ($\pm$0.006)[a]
Cladosporium cladosporioides	0.058 ($\pm$0.006)[a]
Epicoccum nigrum	0.066 ($\pm$0.044)[a]
Penicillium chrysogenum	ND[b]

[a] Parentheses indicate standard errors of mean estimates.
[b] Not determined.

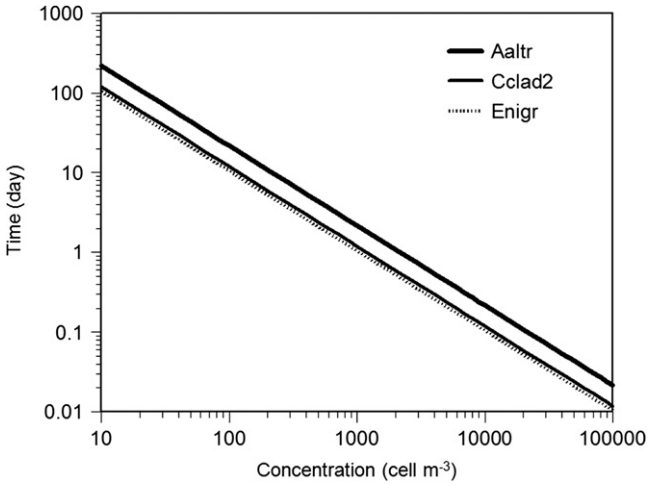

Fig. 3. Time required to collect 100 cells by the passive sampler as a function of airborne fungal concentrations.

5. Summary and conclusions

The present study quantitatively compared airborne fungi concentration measurements by active and passive samplers. The passive sampler is light, small, and silent, making it convenient for personal and indoor air exposure monitoring. The results showed good correlations between these two sampling methods for *A. alternata*, *C. cladosporioides*, and *E. nigrum*, and similar measurement precisions were observed for *A. alternata* and *E. nigrum*. However, the passive sampler was unable to detect *P. chrysogenum*, likely due to its aerodynamic diameter of less than 5 μm. The sampling duration by the passive sampler can be optimized by considering the sampling rates of target fungi and the concentrations in given environments. Because passive samplers are less obtrusive to human subjects, these samplers, in conjunction with growth-independent qPCR methodologies, show potential to improve human exposure studies for airborne fungal pathogen and allergens with aerodynamic diameters greater than 5 μm.

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