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The influence of sampling duration on recovery of culturable fungi using the Andersen N6 and RCS bioaerosol samplers

Abstract The influence of sampling duration on recovery of culturable fungi was compared using the Andersen N6 and the Reuter Centrifugal Sampler (RCS). Samplers were operated side-by-side, collecting 15 samples each of incrementally increasing duration (1–15 min). From 270 samples collected, 26 fungal genera were recovered. Species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Penicillium* and *Ulocladium* were most frequent. Data adjusted to CFU/m^3 were fitted to a Poisson regression model with a logarithmic link function and evaluated for the impact of sampling time on qualitative and quantitative recovery of fungi, both as individual taxa and in aggregate according to xerotolerance. Significant differences between the two samplers were observed for xerotolerant and normotolerant moulds, as well as *Aspergillus* spp. and *Cladosporium* spp. With the exception of *Cladosporium* spp., overall recoveries were higher with the RCS. When the Andersen N6 was used, the recovered levels of *Cladosporium* spp. and unidentified yeasts were reduced significantly at sampling times over 6 min. Similarly, when the RCS was used, recovery of *Aspergillus* spp., *Penicillium* spp., *Ulocladium* spp., unidentified yeasts, and low water activity fungi declined significantly at sampling times over 6 min.

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Practical Implications

Currently, the industry-wide trend for viable air sampling in indoor environmental investigations is to use sampling times between 2 and 4 min in duration. Our results support the routine use of a 6-min sampling time where low spore loads are expected, resulting in improved limits of detection.

Introduction

It is well known that fungal growth is an important contributor to poor indoor air quality (Meklin et al., 2003). Associations between inhalation exposure to indoor fungi and adverse health effects have been suggested (Douwes et al., 2003; Lebowitz et al., 1982; Mandryk et al., 2000; Stark et al., 2003; Wouters et al., 2002); however, the true burden of illness associated with indoor fungal exposure remains uncertain (Institute of Medicine, 2004). Despite this uncertainty, the recognition and elimination of indoor fungal growth and the avoidance of conditions leading to its development have been widely advocated by cognizant bodies and health authorities as prudent measures for health protection [American Industrial Hygiene Association (AIHA), 2005; Environmental Protection Agency (EPA), 2003; Health Canada, 2004; Institute of Medicine, 2004; Thomson and Petticrew, 2005]. Visual inspection combined with viable air sampling has been advocated for the investigation of indoor fungal contamination (Miller et al., 2000).

Several air sampling devices have been widely used in the assessment of buildings for indoor fungal contamination. These devices rely on several methods to collect aerosols, including impaction, centrifugation, filtration and impingement (Henningson and Ahlberg, 1994). Characterization of sampled specimens is carried out by direct microscopy, culture or biochemical

analysis. One widely used device, the Andersen N6 single-stage impactor (Thermo-Electron, Atlanta, GA, USA) (Andersen, 1958; Jones et al., 1985), has long been accepted as the 'gold–standard' method for the evaluation of fungal aerosols (AIHA, 2005; Brachman et al., 1964).

The performance characteristics of the Andersen N6, and that of most other commonly used sampling devices, has been established by theoretical modeling and empirical testing under controlled conditions, with a primary emphasis on particle collection and retention as a function of aerodynamic particle size (Andersen, 1958; Clark et al., 1981; Jones et al., 1985; Macher and First, 1983; Willeke et al., 1998). Little attention, however, has been given to the biological aspects of sampler performance, such as the influence of sampler design, collection conditions and sampling duration on propagule viability.

Long duration samples are preferable to short-term samples as they reduce the analytical detection limit and provide better integration of transient fluctuations in aerospora (AIHA, 2005). However, the increased air volume in long duration sampling may reduce the viability of recovered aerospora due to mechanical damage from shear and impaction forces. Death may also result from osmotic stress arising when evaporation causes a transient reduction in the water activity of the collection medium (Andersen and Cox, 1967; Blomquist et al., 1984; Morris, 1995).

In a study on the effect of air volume on the viability of airborne fungi and bacteria, Durand et al. (2002) used three sets of side-by-side air samples collected at composting facilities on polycarbonate membrane filters for 1 h at 2 l/min. Two of the filter sets were further exposed to a HEPA-filtered air stream for 2 and 5 h, respectively. Although these workers found culture yield to be independent of the length of exposure to the sampler's air stream, their study did not address the possibility that negative effects of sampling duration might occur mainly during the early phase of sampling. Wang et al. (2001), for example, observed an initial reduction in culture recovery during the first few minutes of sampling, with residual viability remaining stable at sampling periods up to 8 h. Similarly, Stanevich and Petersen (1990) observed a significant reduction in recovery when Andersen N6 samples collected for 1 min were compared to those collected for 3 and 5 min. These observations may be due, in part, to physical damage resulting from impaction and continual bombardment from the air stream during sampling (Stanevich and Petersen, 1990; Stewart et al., 1995). Drying and concomitant concentration of the collection medium during sampling may also contribute to cell damage (Macher et al., 1995).

Currently there are no standard measures for biological recovery efficiency, and this aspect of sampler performance is rarely considered in studies comparing sampling methods. The present study investigated the influence of sampling duration on the qualitative and quantitative yield of culturable fungi recovered with the Andersen N6 impactor and RCS samplers in a field performance situation.

Materials and methods

Samplers and media

Viable fungal air samples were collected using a standard RCS (Biotest Diagnostics Corp, Denville, NJ, USA) and an Andersen N6 (Thermo Fisher Scientific, Franklin, MA, USA). The standard RCS is a centrifugal sampler that operates at an effective flow rate of 40 l/min. Samples were collected on proprietary articulated plastic strips filled by the manufacturer with Rose Bengal agar medium containing chloramphenicol (Biotest Diagnostics Corp.). The Andersen N6 is a single-stage jet impactor based on the physical design criteria of the sixth stage of the six-stage cascade impactor (Jones et al., 1985). The sampler was operated using a high volume pump (Gast Inc., Benton Harbor, MI, USA) calibrated to 28.3 l/min using a rotameter (Zefon International, Ocala, FL, USA). Samples were taken on standard 90 mm plastic Petri plates filled with 30 ml Rose Bengal agar medium (Difco; Becton Dickinson, Sparks, MD, USA) (Malloch, 1981) amended with 100 mg/l chloramphenicol (Fischer Scientific Inc., Fair Lawn, NJ, USA).

Eleven sets of samples were collected from buildings at University of Toronto, St. George campus. Rooms with large air volumes and low expected airborne fungal concentrations (e.g. auditoria) were selected *a priori* to reduce the chances of sample overloading. The two instruments were operated side-by-side at a height of approx 1 m to collect 15 samples of 1–15 min duration in random order. To minimize the influence of climatic conditions, sampling was conducted only on precipitation-free days. Samplers were cleaned between each sample set using 70% isopropyl alcohol.

Analysis

All samples were incubated for 10–14 days at 25°C and colonies were enumerated and identified in bright field light microscopy and examined by Nomarski Differential Interference Contrast microscopy, where required. Following the recommendations of Stanevich and Petersen (1990), the 'positive-hole' correction was not applied in the analysis of Andersen N6 samples. Colony characteristics and morphological structures were determined by examining the cultured plates or strips directly under a stereo microscope. Microscopic preparations were made in distilled water, clear lactic acid, or lactofuchsin (Carmichael, 1955) depending on

the organism examined. Fungi with delicate reproductive structures were examined by preparing microscopic mounts on clear adhesive tape placed in mounting fluid and covered with a cover slip (Dring, 1971). Several standard reference works were used during identification procedures, including: Arx (1970), Barnett and Hunter (1986), Barron (1968), de Hoog et al. (2000), Domsch et al. (1980), Ellis (1971, 1976), Hanlin (1990), Malloch (1981), Onions et al. (1981), and Samson et al. (1996).

Data were analyzed on a genus-by-genus basis as well as in cohorts according to water activity (a_w), a measure of the unbound water in the medium available for the growth of the mould (Samson et al., 1996). The genera *Alternaria, Epicoccum* and *Ulocladium* (important components of the typical plant leaf surface fungal community known collectively as 'phylloplane fungi'), colonize high water activity substrata (e.g. a_w: 0.85–0.90) (Domsch et al., 1980). Data for these genera were pooled and analyzed as a 'high a_w' (normotolerant) cohort. *Cladosporium* spp., also typical phylloplane fungi but including some moderately xerophilic species such as *C. sphaerospermum* (Pitt and Hocking, 1999), were observed abundantly in all datasets but were excluded from the cohort analyses to reduce skewing effects. Conversely, species of *Paecilomyces*, *Penicillium* and *Aspergillus* are characteristic of low water-activity habitats (e.g. a_w: 0.70–0.80). Data for these taxa were pooled and analyzed in combination as a 'low a_w' (xerotolerant) cohort.

Datasets for each sampler were compared to determine the influence of sampling duration on recovery in terms of abundance and taxonomic diversity. A trend line was interpolated from each dataset based on a $\log_e$-transformation and fitted to a mixed Poisson regression model using a simple random effects component of random intercept by sampling date. The structure of the covariance matrix was the default in SAS-variance components, using the key assumption that samples collected on the same day were more alike than those collected on different days. Covariates in the model included sampling duration, sampler type and sampler*duration interaction. Variance components were included in the model to account for similarity within sampling day. The influence of sampling duration was inferred from the significance of the sampling duration regression estimate. The difference between the RCS and Andersen N6 samplers was assessed by the significance of the sampler and sampler*duration regression estimates. To examine the influence of sampling duration on recovery, we plotted standardized concentration for each taxon and physiological cohort against sampling duration. We fitted a piecewise horizontal-line mixed Poisson regression model with a change-point at 6 min (chosen by visual inspection), and examined the significance of the regression coefficient for the change-point. P-values < 0.05 were considered significant. All statistical analysis was carried out using SAS v. 9.0 (SAS Institute Inc., Cary, NC, USA). Regression models were fit using Proc GLIMMIX (SAS).

Results

A summary of the taxon frequency for each sampler type is provided in Table 1. Data collected on two of the sampling dates could not be analyzed due to overloading and have been excluded.

Table 1 Summary of fungal taxa observed

Taxon	Frequency (%)*	
	N6	RCS
Acremonium spp.	<1.0	<1.0
Acrodontium spp.	<1.0	–
Alternaria spp.	3.2	15.4
Arthrinium spp.	<1.0	<1.0
Aspergillus candidus	<1.0	<1.0
Aspergillus circumdati group	<1.0	–
Aspergillus flavipes group	<1.0	–
Aspergillus flavus group	<1.0	<1.0
Aspergillus fumigatus	2.3	1.4
Aspergillus nidulans	<1.0	–
Aspergillus niger group	1.5	2.8
Aspergillus ochraceus	<1.0	<1.0
Aspergillus restrictus	<1.0	1.3
Aspergillus spp.	<1.0	<1.0
Aspergillus sydowii	–	<1.0
Aspergillus ustus group	–	<1.0
Aspergillus versicolor group	1.2	9.3
Aspergillus wentii	–	<1.0
Botrytis cinerea	–	<1.0
Chaetomium spp.	<1.0	–
Cladosporium cladosporioides	30.4	20.9
Cladosporium herbarum	9.8	3.3
Cladosporium sp.	1.5	3.2
Cladosporium sphaerospermum	12.9	4.3
Curvularia spp.	<1.0	<1.0
Epicoccum nigrum	<1.0	2.8
Eurotium spp./*Aspergillus glaucus* group	<1.0	<1.0
Fusarium spp.	<1.0	<1.0
Mucor spp.	<1.0	<1.0
Nigrospora spp.	<1.0	<1.0
Paecilomyces spp.	<1.0	–
Paecilomyces variotii	<1.0	–
Penicillium spp.	<1.0	<1.0
Penicillium subgen. *Aspergilloides*	2.2	1.3
Penicillium subgen. *Biverticillium*	5.2	4.6
Penicillium subgen. *Penicillium*	10.7	7.7
Phoma spp.	–	<1.0
Pithomyces spp.	–	<1.0
Ramichloridium spp.	<1.0	–
Rhizopus spp.	<1.0	<1.0
Scedosporium spp.	–	<1.0
Scopulariopsis spp.	<1.0	–
Stachybotrys chartarum	–	<1.0
sterile mycelia	11.3	9.7
Syncephalastrum racemosum	–	<1.0
Tritirachium spp.	<1.0	–
Ulocladium spp.	<1.0	3.2
yeast	1.5	3.9

*% of total colonies observed (N6: n = 1792; RCS: n = 2129).

Table 2 Fungal genera recovered by Andersen N6 Sampler (CFU/m^3)

	Taxon															
	Alternaria spp.		*Aspergillus* spp		*Cladosporium* spp.		*Epicoccum nigrum*		*Penicillium* spp.		*Ulocladium* spp.		Yeasts		Sterile mycelia	
Sampling time (min)	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.
1	11.1	16.7	<LOD	<LOD	44.4	47.1	3.7	11.1	7.4	14.7	<LOD	<LOD	<LOD	<LOD	14.8	24.2
2	3.7	7.3	3.7	3.7	20.4	27.4	1.9	5.6	11.1	11.8	<LOD	<LOD	<LOD	<LOD	11.1	18.6
3	2.5	7.4	1.2	1.2	38.2	40.1	1.2	3.7	4.9	8.1	<LOD	<LOD	<LOD	<LOD	8.6	15.5
4	1.9	3.6	1.9	1.9	46.3	44.9	<LOD	<LOD	9.3	7.7	0.9	2.8	3.7	6.1	10.2	14.9
5	<LOD	<LOD	5.2	5.2	20.7	14.7	<LOD	<LOD	7.4	4.0	1.5	4.4	<LOD	<LOD	3.0	6.8
6	1.9	3.9	2.5	2.5	32.1	23.5	<LOD	<LOD	8.6	8.4	0.6	1.9	2.5	4.0	3.7	5.6
7	1.1	3.1	2.1	2.1	24.3	16.2	<LOD	<LOD	10.6	10.6	<LOD	<LOD	0.5	1.6	5.8	5.7
8	1.9	3.0	1.9	1.9	23.1	15.6	<LOD	<LOD	7.9	6.1	<LOD	<LOD	0.5	1.4	8.8	7.0
9	1.2	2.6	2.9	2.8	25.1	17.8	<LOD	<LOD	11.1	6.7	<LOD	<LOD	1.2	2.6	4.5	5.5
10	0.7	1.5	3.0	3.0	19.3	15.6	<LOD	<LOD	8.5	4.7	<LOD	<LOD	0.4	1.1	4.8	5.8
11	0.8	1.4	1.5	1.5	27.7	22.5	0.8	1.4	8.7	6.4	<LOD	<LOD	0.8	1.4	4.2	3.9
12	1.5	2.5	2.2	2.2	20.4	17.0	<LOD	<LOD	11.4	5.6	<LOD	<LOD	0.3	0.9	5.2	4.9
13	1.3	1.4	0.6	0.6	25.0	24.9	0.6	1.2	8.6	6.8	<LOD	<LOD	0.6	1.2	4.2	4.1
14	2.4	3.1	2.4	2.3	26.5	22.6	<LOD	<LOD	9.0	9.9	<LOD	<LOD	0.3	0.8	4.0	4.6
15	1.7	3.6	3.2	3.2	27.2	15.8	0.2	0.7	8.4	8.7	<LOD	<LOD	0.0	<LOD	7.4	4.7
Overall	2.3	5.9	2.3	4.4	28.1	26.5	0.6	3.4	8.9	8.2	0.2	1.4	0.7	2.3	6.7	10.7

LOD, limit of detection.
**n* = 11.

Table 3 Fungal genera recovered by the RCS (CFU/m^3)

	Taxon															
	Alternaria spp.		*Aspergillus* spp.		*Cladosporium* spp.		*Epicoccum nigrum*		*Penicillium* spp.		*Ulocladium* spp.		Yeasts		Sterile mycelia	
Sampling time (min)	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.
1	11.1	18.2	8.3	25.0	5.6	11.0	<LOD	<LOD	5.6	11.0	5.6	16.7	5.6	11.0	22.2	34.1
2	9.7	10.4	9.7	24.8	20.8	24.2	<LOD	<LOD	8.3	12.5	2.8	5.5	4.2	12.5	4.2	6.3
3	8.3	13.1	17.6	49.7	17.6	25.8	<LOD	<LOD	3.7	8.4	3.7	11.1	4.6	11.1	8.3	11.0
4	3.5	7.1	6.9	18.6	15.3	16.9	0.7	2.1	16.7	20.9	3.4	5.5	<LOD	<LOD	4.9	5.2
5	7.8	9.7	8.9	21.3	17.8	11.5	0.6	1.7	12.2	17.0	1.7	3.5	2.8	5.1	3.9	5.5
6	7.4	7.7	15.7	45.7	14.4	14.9	1.9	4.2	10.2	15.9	0.5	1.4	0.9	2.8	4.6	3.3
7	12.3	10.0	9.5	26.0	18.7	17.1	0.8	1.6	2.4	4.7	2.4	4.0	1.2	1.8	2.8	5.9
8	6.3	5.4	4.5	5.2	18.7	22.1	1.4	2.3	8.3	7.6	0.3	1.0	1.4	3.2	4.2	3.1
9	7.7	6.7	8.6	14.5	19.4	20.4	1.5	4.6	4.9	4.1	1.8	3.1	1.9	2.8	6.2	3.9
10	5.3	4.0	7.8	13.0	13.3	11.9	2.2	5.8	7.5	8.2	0.6	1.1	0.6	1.7	2.5	3.3
11	9.1	7.0	3.1	4.5	14.8	13.0	0.6	1.6	2.8	3.4	0.8	1.7	1.7	3.2	2.6	1.9
12	7.2	7.9	2.3	3.7	12.0	9.4	4.4	13.2	6.5	5.7	0.7	2.1	0.7	1.5	4.4	3.7
13	6.7	6.3	4.6	9.9	16.8	12.0	0.7	1.4	6.0	2.6	1.4	2.7	<LOD	<LOD	3.4	2.9
14	6.9	5.7	7.1	13.1	13.3	11.6	0.8	1.3	4.4	5.5	1.4	1.7	1.2	1.8	4.2	2.7
15	7.6	9.1	6.5	14.6	12.2	15.9	1.1	0.9	6.7	6.3	1.1	1.2	1.5	1.5	6.1	5.0
Overall	7.8	9.0	8.2	22.6	15.4	15.9	1.1	4.2	7.1	10.5	1.9	5.8	1.9	5.6	5.7	10.7

LOD, limit of detection.
**n* = 11.

The most commonly occurring taxa were: *Alternaria* spp. (9.8% of colonies counted), *Aspergillus* spp. (12.1%), *Cladosporium* spp. (42.2%), *Epicoccum nigrum* (1.8%), *Penicillium* spp. (16.3%), sterile mycelia (10.4%), *Ulocladium* spp. (1.9%) and unidentified yeasts (2.8%). Recovery of these genera is common from indoor air (Kuo and Li, 1994; Macher et al., 1991). A break-down of results for the Andersen N6 and RCS are given in Tables 2 and 3, respectively. Thirty-nine taxa were recovered with the Andersen N6, and 38 with the RCS (difference not significant). The two sampling methods recovered 29 taxa in common. Table 4 provides a summary of regression statistics.

Sampling duration did not strongly influence the recovery of *Alternaria* spp. (Figure 1). However, sampling duration influenced recovery for both samplers with certain taxa. The Andersen N6 showed a

Table 4 Summary of regression results

Species/grouping	Intercept β (s.e.)	Intercept P-value	Andersen vs. RCS β (s.e.)	Andersen vs. RCS P-value	Duration β (s.e.)	Duration P-value	Sampler × Duration β (s.e.)	Sampler × Duration P-value
Alternaria spp.	1.922 (0.291)	0.0002	−1.410 (0.451)	0.0020	−0.012 (0.017)	0.5025	−0.016 (0.046)	0.7330
Aspergillus spp.	1.888 (0.453)	0.0031	−1.628 (0.526)	0.0022	−0.074 (0.023)	0.0017	0.062 (0.054)	0.2532
Cladosporium spp.	2.677 (0.314)	<0.0001	0.477 (0.168)	0.0050	−0.028 (0.013)	0.0320	0.006 (0.017)	0.7085
Penicillium spp.	2.185 (0.258)	<0.0001	−0.143 (0.276)	0.6045	−0.045 (0.020)	0.0247	0.054 (0.028)	0.0602
Ulocladium spp.	0.633 (0.497)	0.2381	−0.601 (0.861)	0.4857	−0.086 (0.034)	0.0119	−0.299 (0.161)	0.0644
Yeast	0.835 (0.409)	0.0753	−0.393 (0.564)	0.4863	−0.093 (0.035)	0.0077	−0.043 (0.067)	0.5164
Sterile mycelia	1.602 (0.298)	0.0007	0.333 (0.330)	0.3139	−0.025 (0.024)	0.3046	−0.011 (0.034)	0.7422
High a_w	3.227 (0.258)	<0.0001	0.100 (0.149)	0.5017	−0.024 (0.010)	0.0220	−0.001 (0.015)	0.9717
Low a_w	2.956 (0.237)	<0.0001	−0.706 (0.175)	<0.0001	−0.059 (0.011)	<0.0001	0.063 (0.018)	0.0006
Total colonies	4.044 (0.173)	<0.0001	−0.102 (0.124)	0.4115	−0.036 (0.008)	<0.0001	0.015 (0.013)	0.2388

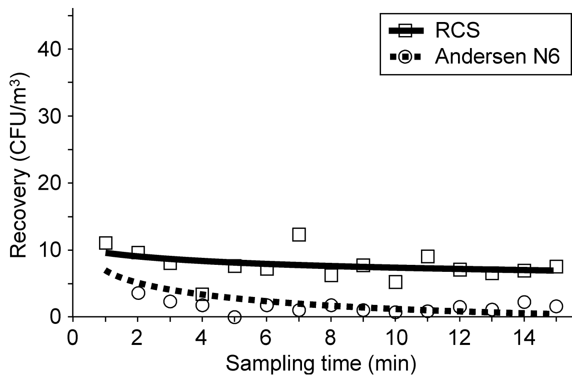

Fig. 1 Average recovery for *Alternaria* spp. Mean recovery as a function of sampling duration (squares/circles), regression model based on $\log_e$-transformation fitted to a Poisson model (line)

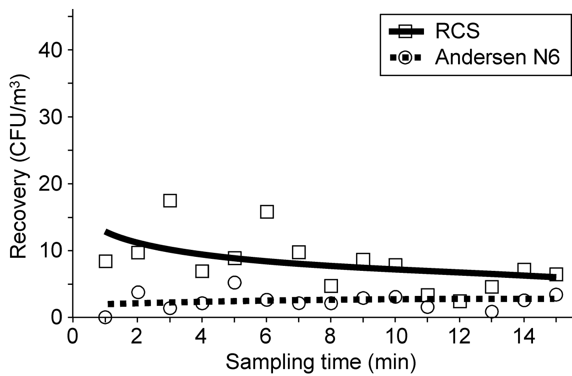

Fig. 2 Average recovery for *Aspergillus* spp. Mean recovery as a function of sampling duration (squares/circles), regression model based on $\log_e$-transformation fitted to a Poisson model (line)

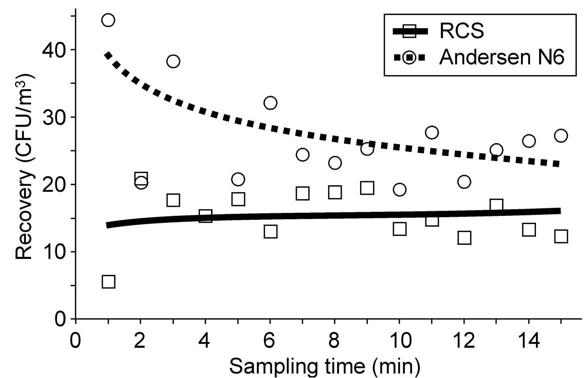

Fig. 3 Average recovery for *Cladosporium* spp. Mean recovery as a function of sampling duration (squares/circles), regression model based on $\log_e$-transformation fitted to a Poisson model (line)

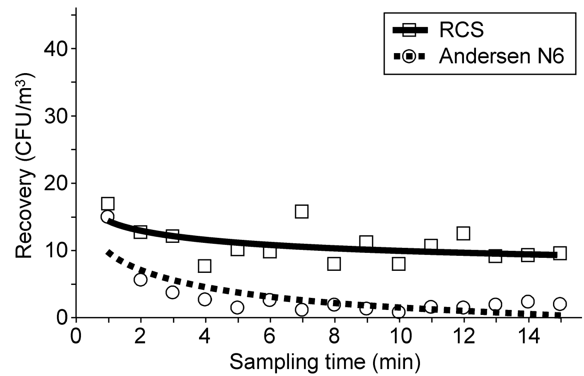

Fig. 4 Average recovery for normotolerant taxa. Mean recovery as a function of sampling duration (squares/circles), regression model based on $\log_e$-transformation fitted to a Poisson model (line)

significantly reduced recovery for *Cladosporium* ($P = 0.0004$) and yeasts ($P = 0.0170$) at sampling durations greater than 6 min. RCS results were more strongly affected by sampling time: at sampling durations greater than 6 min, a significant decrease was seen in recovery rate of multiple organism categories, including *Aspergillus* spp. ($P < 0.0001$), *Penicillium* spp. ($P = 0.0005$), *Ulocladium* spp. ($P = 0.0282$), and yeasts ($P = 0.0129$), as well as the xerotolerant cohort ($P < 0.0001$) (Figures 2–5).

Compared to the RCS, the Andersen N6 showed significantly reduced abundances for normotolerant and xerotolerant cohorts, as well as for *Alternaria* spp. and *Aspergillus* spp, independent of sampling duration;

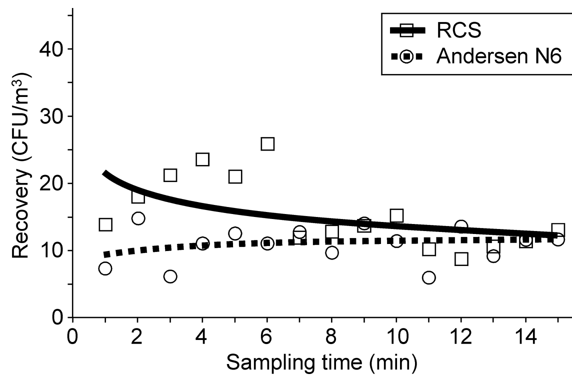

Fig. 5 Average recovery for xerotolerant taxa. Mean recovery as a function of sampling duration (squares/circles), regression model based on $\log_e$-transformation fitted to a Poisson model (line)

however, a significantly increased yield of *Cladosporium* spp. was observed.

In general, with the exception of *Cladosporium* spp., the overall abundance of fungi tended to be greater with the RCS than with the Andersen N6. Recovery curves for the xerotolerant cohort were seen to be strongly similar to one another. The same was true of recovery curves for the normotolerant cohort. There was a strong difference, however, between the xerotolerant recovery pattern and the normotolerant pattern. These results suggest a strong influence of osmotic change on biological collection efficiency.

Discussion

When viable samplers are used, the recovery rates of fungi from air samples depend not only on the physical characteristics of the sampling devices, but also on the device's success in preserving fungal viability during collection. The mechanical collection efficiency of aerosol samplers is described by the d_{50} cutpoint, defined as the diameter of particles collected with 50% efficiency. Typically, this expression does not refer to the physical diameter of particles, but rather, to their aerodynamic equivalent diameter (AED): that is, the diameter of a hypothetical particle of unit density whose terminal settling velocity (TSV) is equal to that of the actual particle. The Andersen N6 has a d_{50} cutpoint of 0.6 μm (Macher, 1989). It is not possible to determine the exact d_{50} cutpoint of the RCS because the air intake and exhaust of the device share the same orifice. Empirical estimates of the d_{50} cutpoint of the RCS range from 5 μm (Clark et al., 1981) to 3–4 μm (Macher, 1989), to below 2 μm (Jensen et al., 1992).

In contrast to physical collection efficiency, the efficiency with which cell viability is preserved (biological collection efficiency) is much more complex, relating to many factors. These include mechanical impaction force, dehydration, rehydration, temperature, oxygen concentration, and ionic strength. Water activity (a_w) is a measure of unbound water available for growth in the medium (Samson et al., 1996). Removal of water from the medium by evaporation increases the solute concentration, thereby reducing the a_w and potentially applying osmotic stress. This effect may occur globally in medium subjected to uniform air conditions, or locally in medium subjected to localized air movement. With fungi, damage due to drying has been shown to be an important contributor to cell injury and death in air sampling (Blomquist et al., 1984; Durand et al., 2002; Wang et al., 2001).

Differences between the two samplers in recovery of several categories is noteworthy. In particular, levels of *Aspergillus* and *Alternaria* were greater with the RCS, while those of *Cladosporium* were greater with the Andersen N6. As well, the Andersen N6 showed significantly poorer recovery of normotolerant and xerotolerant cohorts than the RCS. These trends relate to morphological and physiological characteristics of these taxonomic groups and physiological categories, and how these characteristics influence the mechanical collection efficiency and preservation of cell viability of the organisms, as discussed below.

Aspergillus spp.

Asexual spores (conidia) of members of the genus *Aspergillus* likely serve as the principal airborne dispersal units of these fungi since none of the sexual stages affiliated with this genus have forcibly discharged ascospores (Malloch and Cain, 1972). Conidia of *Aspergillus* spp. are sphaeroidal, and small, typically 2–5 μm diam in physical size. They are borne in dry chains that break apart readily upon disturbance, becoming airborne. However, direct examination of spore trap air samples by microscopy has shown that the dispersal units of these fungi commonly consist of persistent chains of two or more conidia (Madelin and Madelin, 1995).

Our observation of elevated *Aspergillus* recovery by RCS is in conflict with the results of Lee et al. (2004) who noted that the Andersen N6 tended to yield more *Aspergillus* than RCS. They found that samples collected with the RCS also yielded significantly greater overall mean concentrations of these taxa than the other sampling methods tested. Their study also showed that the Andersen N6 used in the outdoor setting had a higher overall recovery than RCS, which in turn tended to have greater average yields indoors. Bellin and Schillinger (2001) similarly found that the Andersen N6 obtained higher yields of *Aspergillus*, *Penicillium* and non-sporulating taxa than another sampling device, the Surface Air System 90 (SAS) (PBI International, Milano, Italy).

Alternaria spp.

Based on spore trap sampling, *Alternaria* spp. are well-documented constituents of the indoor and outdoor aeromycota (Li and Kendrick, 1995). However, the viable fraction of the airborne biomass of these organisms consists of several different cell types, including asexual (in addition to *Alternaria*, some taxa are known to produce *Phoma* synanamorphs) and sexual (*Lewia* spp.) propagules as well as vegetative cell fragments. The relative proportion of each particle type is a function of numerous variables including prevailing environmental conditions and season. Irrespective of the type of viable particle, all may germinate to produce a colony of *Alternaria* sp. Although viable sampling methods do not differentiate between sexual and asexual propagules, the relative contribution of each category is important to both physical and biological collection efficiencies due to differences in morphology, sensitivity to desiccation, and germinability. Conidia of *Alternaria* spp. average $100 \times 17\ \mu m$ in physical dimension (Rotem, 1994). Despite the relatively large physical size of these conidia, their filament-like aspect ratio (l:w > 4) causes them to behave aerodynamically like much smaller particles (Ingold, 1965:15). Empirical estimates have shown the TSV of conidia of *Alternaria* spp. to be between 0.3 and 0.6 cm/s (Gregory, 1973:22). This suggests an AED in the range of 9–13 μm, calculated using the formula of Cox (1995:16) with density assumed to be 1.1 g/cm^3 as given by Miller et al. (1953). Although the ascospores of *Lewia* spp. are physically smaller than conidia of *Alternaria* spp, averaging $20–22 \times 7–9\ \mu m$ (after Simmons, 1986), their AED is similar [10–12 μm AED, based on the calculation described earlier, using TSV data from Ingold (1965:15)]. Both the RCS and Andersen N6 samplers exhibit high collection efficiency for particles above 10 μm AED. As such, given the AED similarity for the two propagule types, the differences in *Alternaria* spp. recovery we observed for the two samplers are unlikely to be due to a bias among spore types arising from differences in mechanical collection efficiency. Most likely they arise instead from superior preservation of cell viability by the RCS.

It has long been suggested that the physical stress of jet impaction in the Andersen N6 may damage cells during sampling (Andersen and Cox, 1967; Blomquist et al., 1984; Morris, 1995). Petri plates sampled in the Anderson N6 and other jet impactors characteristically develop conspicuous dimpling where the agar surface is exposed to the jets. Dimpling is caused by mechanical pressure exerted by air streams as well as by local water loss due to evaporation at the impaction zone. The water loss entails locally increased osmotic tension. Both mechanical and osmotic stress factors probably contribute to a loss in cell viability (Blomquist et al., 1984; Durand et al., 2002; Wang et al., 2001). The more generalized water loss from the collection medium surface with the RCS may have a lower impact on cell viability.

Cladosporium spp.

The significantly high recovery rates of *Cladosporium* spp. with the Andersen N6 compared to the RCS, while intriguing, cannot readily be explained. Species of *Cladosporium* are among the most commonly occurring fungi in phylloplane habitats (Li and Kendrick, 1995). Conidia of *Cladosporium* spp. are globose to elongate, with the longest dimension mostly under 10 μm (Samson et al., 1996). In spore trap samples, *Cladosporium* spp. show a wide range of morphological variation from single conidia to relatively large, non-disarticulated chains.

Unlike many phylloplane fungi, *Cladosporium* spp. may exhibit substantial osmotolerance during active growth (Gunde-Cimerman et al., 2003; Hocking et al., 1994), and ungerminated propagules may tolerate brief periods of even higher osmotic tension (J. Scott, pers. obs.). Pitt and Hocking (1999) refer to the common species *C. sphaerospermum*, in particular, as 'a xerophile, able to germinate and grow slowly at 0.815 a_w'. Recovery of *Cladosporium* spp. by the RCS showed an increasing trend with sampling duration, suggesting a tendency for desiccation to favor *Cladosporium* recovery. The high overall recovery of *Cladosporium* spp. by the Andersen N6 may also, in part, be ascribed to the increased osmotic tension of the impaction zones favoring growth of these osmotolerant taxa. However, the strongly decreasing trend in recovery as a function of sampling duration in the Anderson N6 suggests that osmotic stress alone cannot account for the better overall performance of the Andersen N6 than the RCS with respect to *Cladosporium* spp.

Sampling duration

Sampling duration is an important consideration in the determination of collection efficiency. Short sampling times are associated with increased variability among samples and with results that in general are not representative of the prevailing air microbiota. Longer sampling times are associated with lower limits of detection, and therefore are desirable. Sampling techniques that support long-term samples have been emphasized in recent years (Flannigan, 1997). In the present study, however, increased sampling duration was associated with decreased recovery of most genera collected. With both samplers, this trend was most strongly pronounced at sampling times greater than 6 min. Thus, in situations where low airborne spore loads are expected, sampling times of up to 6 min may be reasonable for both the RCS and Andersen N6, but longer sampling times may yield distorted results.

Our sampling was conducted in buildings with large air volumes to avoid sample overloading. Collecting in spaces with higher spore loads could provide more information about relative fungal aerosol frequencies and differences between samplers. The use of chamber-generated, controlled fungal aerosol would facilitate improved comparison of biological collection efficiencies at different sampling durations.

Summary

Increased sampling duration appeared to decrease the recovery of some taxa of culturable microfungi for both the RCS and Andersen N6 samplers. Sampling durations of up to 6 min did not show significant overall reduction in recovery and may be recommended for air sampling in areas where low airborne spore levels are anticipated. Osmotic change to collection medium during sampling is an important and understudied factor contributing to differences in collection efficiency in viable sampling. Further study is needed to better understand the relationship between sampling duration and the recovery of various biological categories of fungal aerosols.

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