Field Validation of a Novel Personal Cyclone Sampler for Collection of Fungal Spores

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INTRODUCTION

Chen et al. (2004) described a personal aerosol sampler based on cyclone principles that uses a 1.5-ml microcentrifuge tube for particle collection (Figure 1). These authors evaluated the collection efficiency for aerosol particles in the size range of fungal spores for different types of microcentrifuge tubes with and without a polyethylene glycol coating or water. They also compared sampler performance at two airflow rates (2 and 4 L min^{-1}). The microcentrifuge-tube sampler was determined to have an aspiration efficiency of 100% in calm air for particles up to 16 µm. At 4 L min^{-1}, the sampler collected nearly 100% of >3 µm particles and >90% of particles between 2.5 and 3 µm with a 50% cutoff diameter (d_{50}) of 1.5 µm.

The current evaluation includes (i) long-term field trials (collection time: 7–19 hours) at an outdoor location with abundant naturally generated airborne fungal spores during a period of peak release (March–May); and (ii) short- and long-term chamber tests (collection time: 10–40 min and 1.3–5.3 hours, respectively) in a sealed glove box with two types of laboratory-generated airborne fungal spores (*Aspergillus versicolor* and *Scopulariopsis brevicaulis*). Only preliminary results of the field evaluation are reported here.

The overall goal of the project was to evaluate the collection of a personal, cyclone, air sampler relative to widely used environmental samplers (i.e., a filter sampler, another cyclone sampler, and two types of slit impactors) for analysis by advanced techniques, such as polymerase chain reaction (PCR) and immunological assays.

METHODS

Table 1 shows the samplers that were compared in this part of the study. Duplicate NIOSH personal cyclone, reference probe filter, and Burkard slide impactor samples were collected.

Figure 1. Novel cyclone sampler.

The outdoor sampling site was adjacent to a small park with abundant natural and cultivated vegetation. Naturally occurring fungal spores were collected at ~2 m above ground from a 1.2-? 1.4-m platform over a nine-week period (March 26–May 16, 2005).

The two personal cyclone samplers and two filter samplers (each sampling at 4-L min^{-1}) were mounted on a support platform with their inlets aligned horizontally, 12 cm apart. The three higher flow rate samplers sat on one of two railings with the sampling inlets aligned vertically, 40 cm apart. The inlets of the latter samplers were 15 cm below and facing 90° from those of the former samplers. The positions of the samplers on the support platform and railing alternated, as did the positions of the individual samplers within each group to allow as many permutations of sampler placement as possible during 30 trials. Total sampling time ranged from 7 h, 28 min to 19 h, 21 min (mean: 12 h, 26 min; standard deviation: 2 h, 20 min).

Spores were washed from the cyclone microcentrifuge tubes and filters, and ten-fold dilutions were made as needed to obtain countable numbers of spores or colony-forming units (CFUs). Spores were counted and identified with a light microscope for 50 µl of spore suspension spread on a glass slide. Culturable spores were counted and identified in 100 µl of spore suspension spread on malt extract agar incubated at 25°C for 7 days. Concentrations of 12 fungal types were measured in 100 µl of spore suspension by real-time PCR (Zhou et al., 2000). Spores on the slit impactor samples were counted and identified in one horizontal traverse of the entire length of the spore deposit (15–35 mm).

RESULTS

Overall, 31 fungal groups were observed by direct microscope examination and 27 groups by culture analysis. The Burkard cyclone identified the largest variety of spores (23 groups), and the Burkard slide impactor identified the fewest spore types (16 groups). However, no spores collected with the latter sampler were unidentifiable, and *Chaetomium* spp. and *Stachybotrys* spp. were observed on more days by this method.

The Burkard slide impactor gave the highest estimated median and mean spore concentrations and the Burkard cyclone sampler gave the lowest (Table 2). The filter sampler measured the highest single total spore concentration (i.e., highest maximum concentration), the broadest concentration range (highest minus lowest concentration), and had the greatest variation in daily concentration (CV = 86%). Relative to the slit impactor (CV = 26%), the day-to-day variation in fun-

Table 1. Samplers used in field comparison

Sampler	Manufacturer	Flowrate (L min^{-1})	d_{50} (μm)
Novel cyclone	NIOSH	4.0	1.5
Reference filter	NIOSH	4.0	—
Slit impactor	Burkard Manufacturing Co.	10.0	5.2
Reference cyclone	Burkard Manufacturing Co.	16.6	1.2

Table 2. Summary statistics for total concentration (spores m^{-3}) (N = 30)

	Burkard slide	Filter	Burkard cyclone	Novel cyclone
Min	2 080	370	118	487
Med	3 730	2 210	279	1 900
Max	6 220	12 200	977	8 200
Mean	3 790	3 120	341	2 230
SD	1 000	2 680	200	1 510
CV	26%	86%	59%	68%

gal spore concentration also was higher for the two cyclone samplers that were analyzed by resuspension of spores prior to counting (CV = 59% and 68%). Figure 2 shows one comparison of the concentrations of the major fungal spore types.

CONCLUSIONS

All samplers showed agreement on the major types of spores present in outdoor air: Basidiospores 66%–79%; Ascospores 5%–15%, *Cladosporium* spp. 2%–16%, and *Aspergillus/Penicillium* spp. 2%–8% of total spore concentration. The Burkard slide impactor estimated the highest mean ambient spore concentration and had the lowest day-to-day concentration variation (mean: 3 790 spores m^{-3}; CV: 26%) but identified the fewest spore types (16 groups). However, no spores were unidentifiable on the Burkard slides, and *Chaetomium* spp. and *Stachybotrys* spp. were observed on more days by this method (10 and 16 days, respectively, versus 0 or 1 days for the other three samplers).

The reference filter and novel cyclone samplers estimated intermediate air concentrations (mean: 3 120 and 2 230 spores m^{-3}, respectively), but concentration variation was highest for the filter sampler (CV: 86% and 68%, respectively). These samplers recovered 21 and 19 spore types, respectively. The Burkard cyclone sampler estimated the lowest air concentration (mean: 340 spores m^{-3}; CV: 59%), but identified the most spore types (23 groups).

Paired *t*-tests identified no significant difference between mean air concentrations measured by the Burkard slide impactor and the NIOSH reference filter but significant (p = 0.04) to highly significant (p <0.0001) differences between the other sampler pairs. The two cyclone samplers showed the highest correlation between total spore concentrations (correlation coefficient = 0.76) and the second highest correlation was that between the novel cyclone and the Burkard slide impactor (0.65). All other correlations were below 0.60 (0.51–0.58). The slopes of the paired concentration measurements were closest to unity between the Burkard slide impactor and the novel cyclone (0.97) and reference filter (1.55) samplers. The convenience of a personal sampler that collects into a microcentrifuge tube was demonstrated under representative sampling condition, and the novel cyclone performed well relative to conventional devices.

Keywords: *Bioaerosol, Cyclone, Fungi, Personal Exposure*

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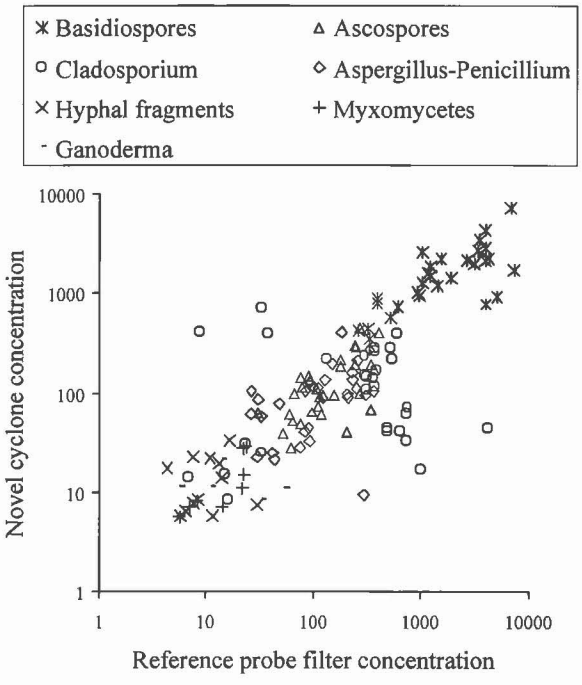

Figure 2. Filter and novel cyclone sampler comparison.

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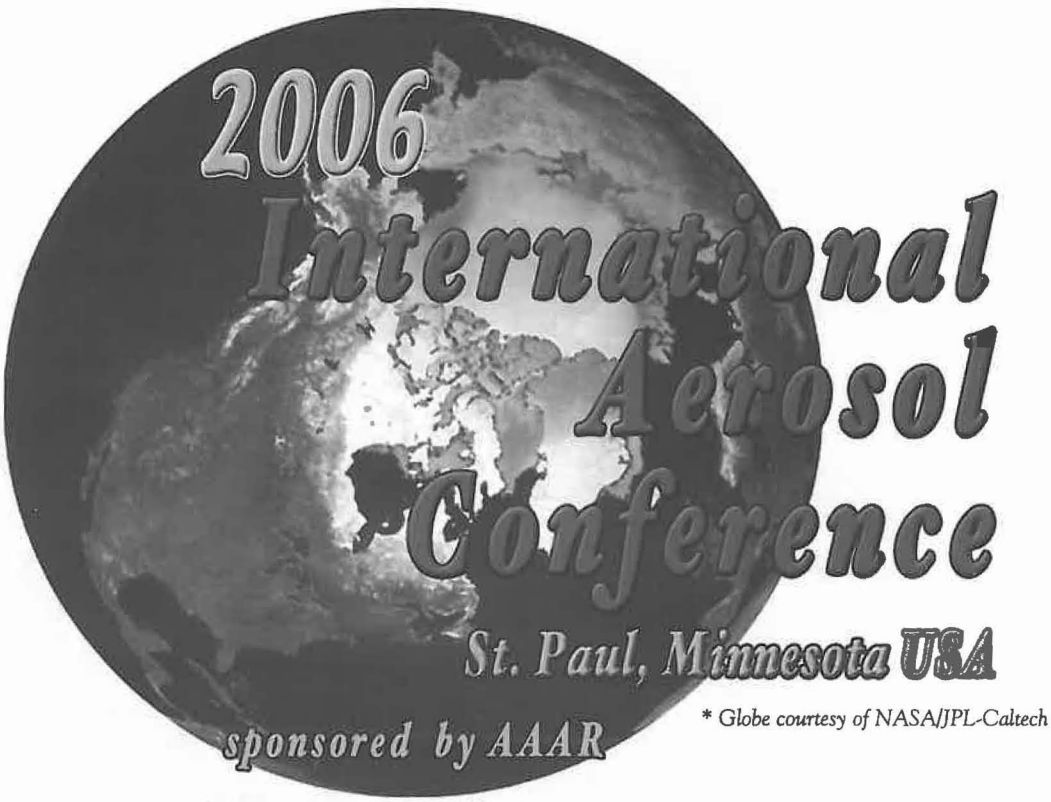

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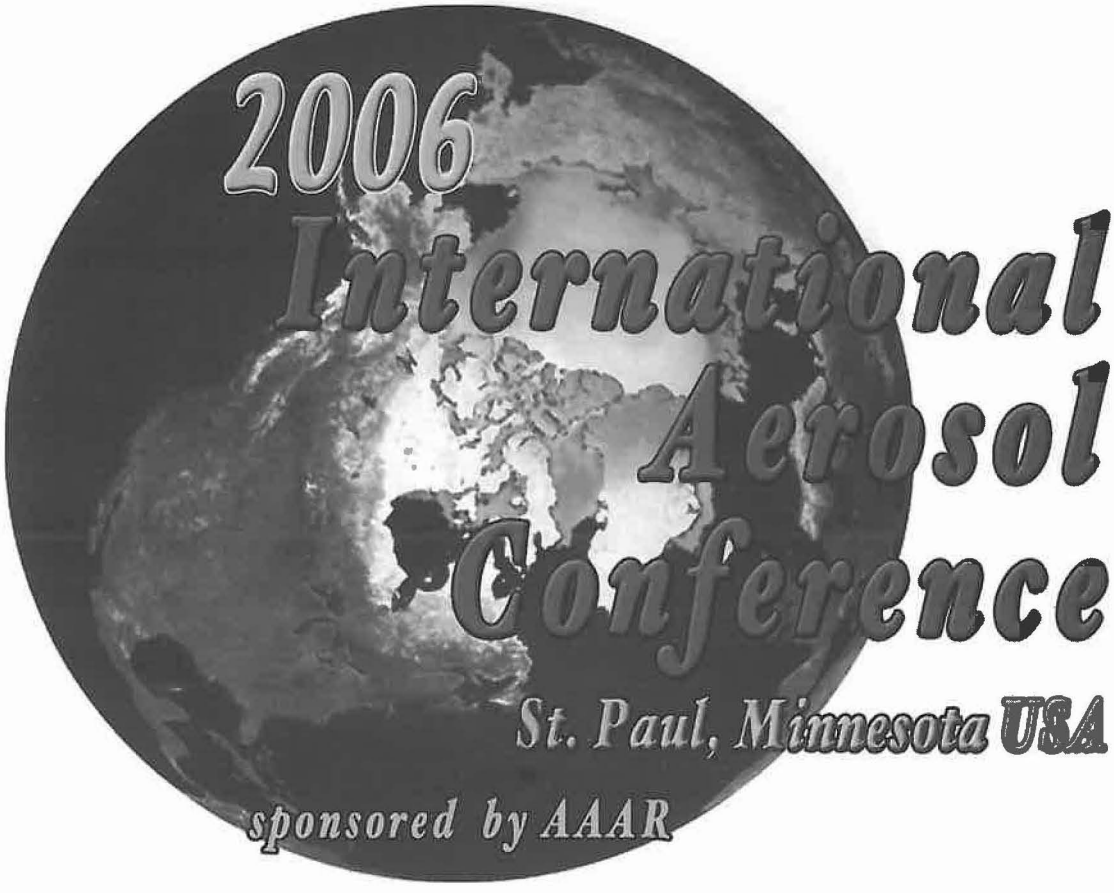

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