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Atmospheric Environment 37 (2003) 4723–4733

ATMOSPHERIC ENVIRONMENT

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Performance of the Button Personal Inhalable Sampler for the measurement of outdoor aeroallergens

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Received 19 March 2003; accepted 14 August 2003

Abstract

No personal aerosol sampler has been evaluated for monitoring aeroallergens in outdoor field conditions and compared to conventional stationary aerobiological samplers. Recently developed Button Personal Inhalable Aerosol Sampler has demonstrated high sampling efficiency for non-biological particles and low sensitivity to the wind direction and velocity. The aim of the present study was to evaluate the Button Sampler for the measurement of outdoor pollen grains and fungal spores side-by-side with the widely used Rotorod Sampler. The sampling was performed for 8 months (spring, summer and fall) at a monitoring station on the roof of a two-storied office building located in the center of the city of Cincinnati. Two identical Button Samplers, one oriented towards the most prevalent wind and the other towards the opposite wind and a Rotorod Sampler were placed side-by-side. The total fungal spore concentration ranged from 129 to 12,980 spores m^{-3} (number per cubic meter of air) and the total pollen concentration from 4 to 4536 pollen m^{-3}. The fungal spore concentrations obtained with the two Button Samplers correlated well ($r = 0.95$; $p < 0.0001$). The pollen data also showed positive correlation. These findings strongly support the results of earlier studies conducted with non-biological aerosol particles, which demonstrated a low wind dependence of the performance of the Button Sampler compared to other samplers. The Button Sampler's inlet efficiency was found to be more dependent on wind direction when sampling larger sized Pinaceae pollen grains (aerodynamic diameter $\approx 65\,\mu m$). Compared to Rotorod, both Button Samplers measured significantly higher total fungal spore concentrations. For total pollen count, the Button Sampler facing the prevalent wind showed concentrations levels comparable to that of the Rotorod, but the Button Sampler oriented opposite to the prevalent wind demonstrated lower concentration levels. Overall, it was concluded that the Button Sampler is efficient for the personal sampling of outdoor aeroallergens, and is especially beneficial for aeroallergens of small particle size.

Keywords: Personal sampling; Pollen; Fungal spore; Button Sampler; Aeroallergens

1. Introduction

Sampling of outdoor aeroallergens is challenging due to the wide size range of the particles and the variation in the samplers' inlet efficiency caused by changing wind conditions. The importance of monitoring outdoor aeroallergens, especially pollen grains and fungal spores in relation to the prevalence of respiratory allergy and asthma has been established by many researchers (Chapman, 1999; D'Amato et al., 1998; Solomon, 2002). This information is important for the clinicians when selecting the proper antigenic extract of pollen or

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doi:10.1016/j.atmosenv.2003.08.021

molds for skin-prick tests of allergy patients. The information is also beneficial to the allergy patients enabling them to take necessary precaution measures in advance during the peak pollen and mold days.

To assure an adequate assessment of aeroallergen exposures and to understand the relationship between exposure and health effects, the accurate measurement of personal exposure to aeroallergens should be more advantageous than the traditional area sampling by a stationary sampler. Several studies have reported that the level of allergenic fungal spores measured with stationary aerosol samplers was not elevated while the tested indoor environments were visibly contaminated with mold and allergic symptoms were present among the occupants (e.g., Su et al., 2001; Verhoeff and Burge, 1997). This discrepancy indicates a poor correlation between the measured aeroallergen concentration and consecutive health effects. In their recent study, Toivola et al. (2002) detected higher concentrations of viable airborne fungal spores in personal inhalable samples compared to stationary samples suggesting that short-term stationary measurement underestimates the real personal exposure to fungal spores. Previously, few personal samplers have been tested for measuring bioaerosols including aeroallergens. For example, Rautiala et al. (1998) used 37-mm cassettes for the personal sampling of fungal spores; Kenny et al. (1999) tested a modified IOM sampler for the measurement of bacteria and fungi, and Agranovski et al. (2002) proposed a porous medium submerged in a liquid layer for the personal sampling of fungal spores and bacteria. In addition, Graham et al. (2000) proposed a nasal sampler for inhaled aeroallergens. Their approach, however, seems to be suitable only for short-term sampling, which may not adequately reflect true exposure.

All the above-mentioned studies have been performed in indoor environments and none of these included pollen. The personal inhalable sampling of pollen, however, also seems to be a better measure of human exposure than the stationary one. Furthermore, pollen grains are considerably larger in particle size than other aeroallergens as some are around 100 μm. The ACGIH/CEN/ISO inhalable sampling convention (American Conference of Governmental Industrial Hygienists, 1999; Comite Europeen de Normalisation, 1993; International Organization for Standardization, 1995) suggests that the sampling efficiency should be about 50% for particles in the 40–100 μm size range (not 100%). The sampling efficiency of a personal inhalable aerosol sampler should closely simulate this convention and demonstrate low dependence on wind velocity and direction.

The outdoor aeroallergen concentrations may vary in different locations of the same city or rural area depending on the proximity of vegetation or different flowering time of the plants (Soldevilla et al., 1995; Sen, 1999). Frenz et al. (1997) collected 122 samples during 7 months at two locations 5.6 km apart and found no statistically significant difference between locations in the average total pollen concentrations. The daily pollen concentrations, however, determined in the same two locations showed a difference of >100 grains m^{-3} in approximately half of the samples. This difference was observed frequently on the days when airborne pollen concentration exceeded 100 grains m^{-3}. Soldevilla et al. (1995) reported that the airborne concentration measured at the heights of 1.5 and 15 m did not differ for several pollen types, such as Chenopodiaceae, *Olea, Plantago* and Poaceae ($p > 0.2$). This study did report a significant difference for Urticaceae pollen ($p < 0.01$) measured at those heights. Leuschner (1999) found a significant difference for total pollen concentration between two samplers placed at ground level and at 30 m height (percentages of occurrence were 99.1 and 77.6, respectively). When *Quarcus* and *Pinus* counts were excluded from the total concentration, however, no significant difference was observed (the corresponding percentages of occurrence were 67.9 and 67.8). Lyon et al. (1984) compared the concentration of four fungal spore types collected at heights of 1.5, 9 and 30 m and found that the data obtained at 9 and 30 m were not significantly different. A significant difference, however, was detected for *Cladosporium* and Basidiospores at 1.5 m compared to 9 and 30 m, particularly when more moisture was present in the air. Chakraborty et al. (2001) found an inverse correlation ($r = -0.83$ to -0.99) between the airborne concentration and the height for the pollen grains of Poaceae, Cyperaceae, Cheno-Amaranthaceae, Asteraceae and fungal spores of *Alternaria, Curvularia, Fusarium* and *Drechslera* collected between 1 and 6 m. Due to the above-described variability and the fact that aeroallergens cover primarily inhalable particle size range of 1–100 μm, we conclude that the personal inhalable sampling of outdoor aeroallergens is important for aeroallergen exposure assessment. However, it appears that no personal inhalable sampler has previously been tested under outdoor field conditions side-by-side to a standard aerobiological sampler for a long sampling period.

The Button Personal Inhalable Sampler (SKC, Inc., eighty-four, PA) is a personal inhalable sampler with a curved porous inlet. The sampling efficiency of this sampler follows well the ACGIH/CEN/ISO inhalability convention (Aizenberg et al., 2000a). The sampler has low dependence on wind direction and velocity for particles $\leqslant 70$ μm aerodynamic size. In laboratory conditions, the sampling efficiencies of the Button Sampler determined in a stationary and personal mode were about the same (Aizenberg et al., 1998). Due to this important feature, a stationary sampler evaluation setting can be used for predicting its suitability for the personal sampling. Moreover, as a filter collector, the

Button Sampler is efficient for smaller particles and can be used with different filter materials in conjunction with different analysis methods, such as microscopic counting, immunochemical, biochemical, and molecular biological analysis.

The Rotorod Sampler® (Sampling Technologies, Inc., Minnetonka, MN) (Grinnell et al., 1961) is an impaction sampler with a rotating arm having two transparent plastic rods coated with silicon grease, or other adhesives. Airborne fungal spores and pollen, which are suspended in the atmosphere, are collected on the collector rods by impaction and interception. The collected pollen and fungal spores on the surface are analyzed by light microscopy. The Rotorod Sampler is well known as one of the most common methods for the collection of outdoor aeroallergens in the United States as well as in the other countries of the world. Frenz and Lince (1997) reported that approximately 300 Rotorod samplers are presently operating for routine aeroallergen monitoring in the United States. Consequently, most of the available reports on aeroallergens in the United States are based on the measurements conducted using this sampler. The Rotorod has been selected in the present study to represent a standard sampling technique for outdoor aeroallergen monitoring. The objectives of the present study were to investigate the feasibility of the Button Sampler for collecting inhalable outdoor aeroallergens up to 100 μm and to compare the performance of the Button Sampler with a standard Rotorod Sampler.

2. Methods

2.1. Description of the samplers

2.1.1. The Button Personal Inhalable Sampler

The Button Sampler includes: (1) a curved porous inlet, (2) a collection filter, (3) a porous screen to hold the filter below the inlet, (4) an air outlet below the filter holder connected with a personal sampling pump.

The inlet is made by a portion of a spherical shell with evenly placed numerous orifices of 381 μm diameter. The total porosity of the inlet is 21%. It has a subtended angle of 160° resulting in a screen area of 19.6 cm^2. The curved symmetrical geometry of the inlet reduces the effects of wind velocity and direction on the sampling efficiency and results in uniform particle deposition on the filter. The blunt semi-spherical shape of the inlet reduces turbulence effects caused by flow separation and thus decreases the airflow disturbance in the vicinity of the sampler. The size of the orifices and the inlet porosity were chosen to assure that the aspiration efficiency of the Button Sampler decreases monotonically with the particle size, following the inhalability convention. The position of the 25-mm diameter filter holder directly behind the inlet minimizes the particle transmission losses. Since a periphery of the filter is covered with a 1.5-mm sealant o-ring, a filter portion of 22-mm diameter (area = 380 mm^2) is available for the particle deposition.

In the present study, mixed cellulose ester (mixture of cellulose acetate and cellulose nitrate) membrane filter of 1.2 μm pore size (Millipore Corp., Bedford, MA) was used for sampling of airborne pollen and fungi because this material can be cleared for light microscopic analysis. Samples were collected at a flow rate of 4 l/min continuously for 24 h using a heavy-duty pump (Model: 1531-107-0288, Gast Corp., Benton Harbor, MI). After sampling, each Button Sampler was covered with a clean cap and carried carefully to the laboratory in a horizontal position using a dust free box. The filter was placed on a glass slide and cleared by acetone vapor using a modified instant acetone-vaporizing unit (Model: Quickfix, Environmental Monitoring Systems, Charleston, SC). The resulting sample was mounted using glycerin jelly (Gelatin: 20 g, Phenol crystals: 2.4 g, Glycerol: 60 ml, Water: 70 ml) mixed with Calberla's stain and covered with a square 25 × 25 mm^2 cover glass. When the mounting jelly became solidified the edge of the cover glass was sealed by transparent nail enamel. The samples were analyzed for pollen grains and fungal spores using light microscope as described below in Section 2.3.

2.1.2. The Rotorod Sampler

In the Rotorod Sampler, the aeroallergens are collected using two clear polystyrene rods with the following dimensions: width = 1.52 mm, length = 1.52 mm, height = 32 mm. The rods are rotated by an electric motor at 2400 rpm around a fixed circumference (in contrast to the old models of the Rotorod Samplers where the circumference radius increased at the beginning and decreased at the end of the sampling). Prior to sampling, the wind-facing side of the rods was coated with fresh silicone grease in a clean place following the manufacturer's operating instructions (Brown, 1993). After sampling, the rods were transported to the laboratory in dust proof transit vials, placed in a special slide with grooves, and stained with Calberla's stain. The fungal spores and pollen grains were counted by light microscopy (see Section 2.3).

2.2. The sampling site and position of the samplers

The rooftop of a two-storied office building about 3 miles north of the downtown Cincinnati was selected for sampling. The height of the rooftop was about 7 m. The nearby vegetation was sparse and there were no tall buildings in the proximity allowing free movement of wind and spatially uniform intensity of the solar radiation at the sampling location.

The Rotorod Sampler was installed on a stand placed on the roof about 2 m away from the margin of the roof. Two Button Samplers fixed onto a sampling tripod about 7.5 cm below a rain shield were placed about 1.5 m away from the Rotorod Sampler at the same distance from the roof margin. The position of the Button Samplers was vertical to ground level and they were placed back to back so that the two inlets were oriented 180° opposite to each other: one towards southwest (SW) direction and the other towards northeast (NE). Based on the measurements at the meteorological station at the Cincinnati northern Kentucky international airport, the most prevalent wind directions in Cincinnati were SW and SWW: the wind was blowing from these directions during 13.6% and 14.0% of the sampling hours, respectively. In contrast, the NE direction occurred only during 3.6% of the sampling hours (as reported by the National Climatic Data Center, Ashville, NC).

The sampling began in October 2001, discontinued for the months of winter (November–February) and continued again in March 2002 until the end of September 2002. Both Button Samplers were operated for 24 h continuously from Sunday to Friday starting every morning at about 8:30 AM. The Rotorod Sampler was also operated for the same duration of time. The Rotorod Sampler's timer was adjusted to the standard 10% duty cycle (1 min in the 'on' position followed by 9 min in the 'off' position).

2.3. Microscopic counting of pollen grains and fungal spores

When the fungal spores and pollen were collected with the Button Sampler, the count was performed on 40 randomly selected microscopic fields using a Nikon (Labophot 2, Nikon Corp., Japan) high-resolution light microscope. For most samples the magnification of 100× was used for the pollen enumeration, except the cases when the grains were not identifiable or the deposition was too dense (the magnification of 400× was applied in these cases). The fungal spore enumeration was always performed at 400×. Phase contrast objectives were used to identify unpigmented hyaline spores.

The total pollen or fungal spore count, $N_{\text{total(BUTTON)}}$, on the filter was calculated as follows:

$$N_{\text{total(BUTTON)}} = N_{\text{f}} \times A_{\text{total(BUTTON)}}/A_{\text{f}}, \quad (1)$$

where N_{f} is the average number of spores/pollen per microscopic field, $A_{\text{total(BUTTON)}}$ is the total area of the filter (380 mm^2) and A_{f} is the area of a microscopic field (0.1452 mm^2 at 400× magnification and 2.19 mm^2 at 100× magnification).

The flow rate of the Button Sampler operating in the personal sampling mode is 4 l/min. Following the quality assurance protocol, the sampling flow rate was measured by a DryCal® DC-Lite Calibrator (SKC, Inc., eighty-four, PA) before and after each 24-h sampling. The average flow rate, F, from these two measurements, the sampling time, t, and the total pollen/fungal count, $N_{\text{total(BUTTON)}}$, were used to calculate the total airborne concentration, C_{BUTTON} (fungal spores: spores m^{-3}; pollen grains: pollen m^{-3}) as follows:

$$C_{\text{BUTTON}} = N_{\text{total(BUTTON)}}/(F \times t). \quad (2)$$

When samples were taken with the Rotorod Sampler, the particle enumeration was performed by a Zeiss polarized light microscope (Carl Zeiss, Germany) either on the entire deposition area of the rods' surface (34.32 mm^2) when the particle surface density was low enough for enumeration or on the microscopic scanning lanes along the width of the rod (60 small segments of a porton graticule × width of the rod = 0.6 mm × 1.56 mm = 0.99 mm^2), when it was too high. The total volume of the air encountered by the rods of the Rotorod Sampler, V was calculated as follows (Brown, 1993):

$$V = W \times H \times D \times \pi \times \text{RPM} \times t, \quad (3)$$

where W is the rod width, H is the rod height, D is the head diameter, RPM is the rotations per minute (2400 min^{-1}). The total number of particles collected by the Rotorod Sampler was determined as follows:

$$N_{\text{total(ROTOROD)}} = N_{\text{SL}} \times A_{\text{total(ROTOROD)}}/A_{\text{SL}}, \quad (4)$$

where N_{SL} is the average number of particles per microscopic scanning lane, $A_{\text{total(ROTOROD)}}$ is the total area of pollen deposition on the rod, and A_{SL} is the area of a microscopic scanning lane along the width of the rod.

The conversion of the count into airborne concentration, C_{ROTOROD} (fungal spores: spores m^{-3}; pollen grains: pollen m^{-3}) was done following the manufacturer's operating instructions (Brown, 1993) as follows:

$$C_{\text{ROTOROD}} = N_{\text{total(ROTOROD)}}/V. \quad (5)$$

Pollen grains and fungal spores from all samples were identified up to the genus, family, or group level based on their morphological characteristics. Identification was based on reference slides (Aerobiology Instruction and Research, Brookline, MA) and on the illustrated identification manual by Smith (1990). The morphologically indistinguishable and unidentifiable spores below 5 μm in size (at 400× microscopic magnification) were counted into a separate group referred to as 'spores < 5 μm'.

The aerodynamic diameter (AD) of four selected pollen grains and fungal spores was calculated from their microscopic sizes following the standard formula (Hinds, 1999) and assuming that the particle density was 1 g cm^{-3}.

2.4. Statistical analysis of data

The total concentrations of pollen grains and fungal spores were found to be lognormally distributed, as determined by the Shapiro–Wilk test. Geometric means (GM) and coefficients of variance (CV) were used to characterize total pollen and total fungal spore concentrations for each sampler, where $GM = \exp(\bar{C})$, $\bar{C}$ =arithmetic mean of log transformed data, and CV = standard deviation of log-transformed data divided by the GM. When subgroups of pollen and fungal spores (particles of four different aerodynamic sizes) were analyzed separately the data were found not to follow lognormal distribution. Therefore, pollen and fungal subgroups were characterized using the median and range of the data. Because all samplers were operated in parallel at the same place for the same duration of time, paired tests (*t*-test for total fungal spores and pollen, Wilcoxon signed rank test for subgroups) were conducted to study the differences between the two Button Samplers operated side-by-side (SW and NE), as well as the differences between each Button Sampler and the Rotorod Sampler. Regression analyses were also carried out to estimate the lines fitted to pollen and fungal spore total concentrations for the Button Samplers, and the Rotorod versus each Button Sampler. Repeated measures analysis of variance of weekly median counts was carried out to detect seasonality patterns of total fungal spores and pollen grains. Data from SW and NE Button Samplers were analyzed for all statistical analyses, and when these were not significantly different, the data were combined while compared to the Rotorod Sampler.

All statistical analyses were done using SAS software (version 8.02 for Windows; SAS Institute, Inc., Cary, NC).

2.5. Quality control of sample analyses

To estimate laboratory variability in the microscopic analysis of pollen grains and fungal spores, 7% randomly selected samples obtained by both the Button Samplers and the Rotorod Sampler were analyzed in duplicate independently by two researchers. The average and standard deviations (SD) of the ratios of the data obtained by two researchers were used to find out the subjective variability of the data.

3. Results and discussion

A total of 513 samples were collected from all samplers. Pollen analysis was performed for 500 samples as 3% ($n = 13$) were lost due to rain, disorder in the pumps or sampling set-up or during sample handling. There were only 444 samples analyzed for fungal spores as an additional 11% ($n = 56$) of fungal spore samples were lost due to excessive concentration of dust particles that limited the accuracy of the fungal spore enumeration.

A total of 28 fungal genera/groups and 19 pollen genera/families were recorded from the samples obtained with the Button Samplers. The range of total fungal spore concentrations was 829–12,980 spores m^{-3} during fall (September and October), 129–4654 spores m^{-3} during spring (March–May), and 951–12,562 spores m^{-3} during summer (June–August). The total pollen concentration ranged from 4 to 181 pollen m^{-3} during fall, from 3 to 4536 during spring, and from 1 to 210 pollen m^{-3} during summer. Among fungal spore types, we found that 'spores $< 5\,\mu m$' group, *Cladosporium, Alternaria, Epicoccum*, Ascospores, Basidiospores, and Smut spores were dominant. The dominant pollen types were: *Ambrosia* (Ragweed), Poaceae (Grass), *Quarcus* (Oak), *Juglans* (Walnut), *Juniperus* (Juniper), *Betula* (Birch), *Ulmus* (Elm), *Salix* (Willow), and Pinaceae (Pine). Repeated measures analysis of variance of weekly median pollen and fungal spore concentrations showed that the pattern of seasonal variability was the same across samplers. Means of the weekly median concentrations for fungal spores across samplers in spring, summer and fall were 1385, 4131, 4413, respectively. The concentrations differed significantly between spring and summer ($p = 0.0001$), and spring and fall ($p = 0.0003$). Means of the weekly median pollen concentrations across samplers for spring, summer and fall were 71, 12, and 34, respectively. The concentrations differed significantly between spring and summer only ($p = 0.002$) (results not shown).

3.1. The Button Sampler orientation effect: the data obtained with two Button Samplers (one oriented SW and the other NE)

The regression plots of the data from two Button Samplers oriented in two directions (SW, the most prevalent wind direction and NE, opposite to SW) are presented in Fig. 1: Fig. 1a for fungi and Fig. 1b for pollen grains. The value of r^2 in the regression is 0.92 for fungal spores and 0.77 for pollen grains, indicating a high degree of concordant variability (92% and 77%). The regression coefficients for both fungal spores and pollen grains ($r = 0.95$ and 0.88, respectively) indicate a positive correlation between the data from the two Button Samplers. As *p*- values are < 0.001 in both cases, these regression formulae can be used to estimate concentrations of pollen grains or fungal spores for the Button Sampler facing the wind from the data obtained by the Button Sampler facing the opposite direction, and vice versa.

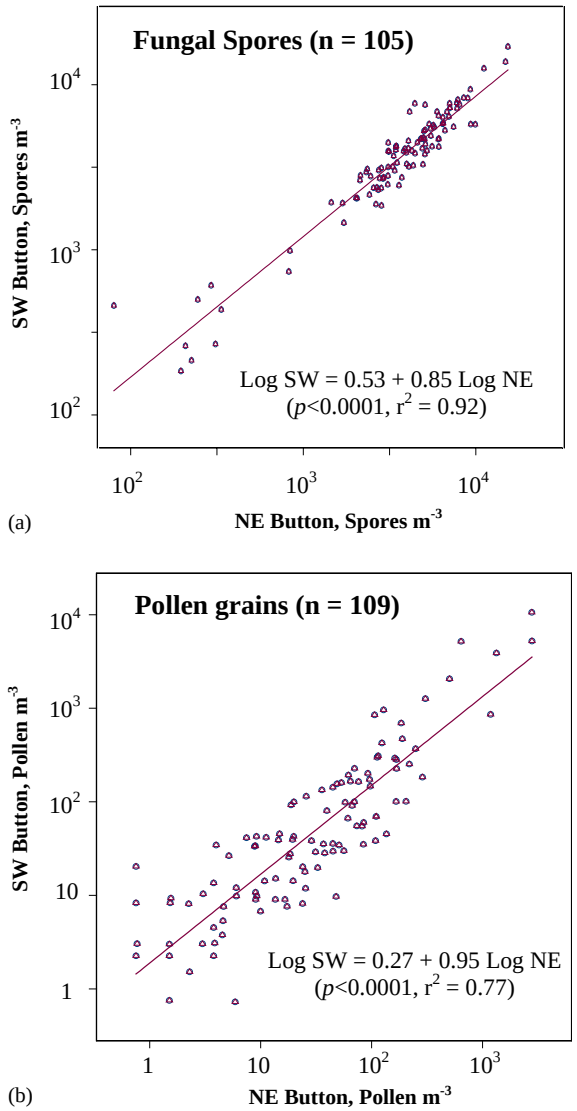


Fig. 1. Regression analysis plot for the log-transformed data on airborne concentrations of fungal spores (spores m^{-3}) (a) and pollen grains (pollen m^{-3}) (b), measured by the two oppositely oriented Button Samplers (SW = sampler oriented towards SW, NE = sampler oriented towards NE).

GM, CV, and numbers of observations for the two Button Samplers are presented in Table 1. Overall, 105 fungal samples and 109 pollen samples were analyzed. Results of paired *t*-tests showed that for fungal spores, no statistically significant difference was observed between the bio-particle concentrations determined with the two Button Samplers, one oriented SW and the other NE. For pollen grains, however, the SW Button Sampler showed significantly higher particle concentration than the NE Button Sampler. Due to their smaller size and hence lower inertia, fungal spores (in average) are not affected by the wind direction as much as pollen grains and thus the sampling efficiency of the Button Sampler collecting fungal spores is less dependent on its orientation. Pollen grains are larger and have higher inertia; thus, wind direction had a significant role in the performance of the Button Sampler as the sampler oriented towards the prevalent wind collected more pollen. Earlier study by Aizenberg et al. (2000a), conducted with non-biological particles, has also shown that the Button Sampler is less sensitive to wind direction for the particles of smaller sizes and becomes more sensitive when the particle size is larger (70 μm).

3.2. Performance of the Button Sampler for the bio-particles of different sizes: the genus/family/group-specific data obtained with two Button Samplers (one oriented SW and the other NE)

Overall, 19 pollen genera/families and 28 fungal genera/groups were identified from the filters obtained with the Button Samplers. After the filters were analyzed for specific bio-particles, two types of pollen grains and two types of fungal spores of different aerodynamic sizes were chosen for the genus/family/group specific enumeration, and their airborne concentrations were determined. The two selected fungal spore types belong to the group referred as 'spores $<5\,\mu m$' ($AD \approx 2\,\mu m$) and *Alternaria* ($AD \approx 18\,\mu m$). The two selected pollen types were *Ambrosia* ($AD \approx 24\,\mu m$) and Pinaceae ($AD \approx 65\,\mu m$). The aerodynamic sizes (calculated using Hinds, 1999) represent the particles that deposit primarily in tracheobronchial region ('spores $<5\,\mu m$'

Table 1
Paired *t*-test comparison between the data obtained with two Button Samplers (SW and NE) measuring the total concentrations for fungal spores (spores m^{-3}) and for pollen grains (pollen m^{-3})

Bio-particle type	SW Button (facing SW, the prevalent wind)		NE Button (facing NE, the opposite wind)		*p*-Value (two-sided)
	GM (spores/pollen m^{-3})	CV	GM (spores/pollen m^{-3})	CV	
Fungal spores ($n = 105$)	3374	1.0	3364	1.2	0.92
Pollen grains ($n = 109$)	43	6.5	27	4.9	<0.001

group), nasopharyngial region (*Alternaria* and *Ambrosia*) and nasal region (Pinaceae) of respiratory system during inhalation. Thus, these cover the lower, intermediate, and upper ranges of the inhalable aerosol fraction.

Table 2 presents the ranges of aeroallergen concentration and the median values of their distributions for the group of 'spores $<5\,\mu m$' ($n = 105$), *Alternaria* ($n = 69$), *Ambrosia* ($n = 35$) and Pinaceae ($n = 31$). Statistically significant difference ($p = 0.05$) was observed in the case of Pinaceae pollen only, which was the largest pollen grain among the dominant pollen grains. Similar to the data presented above for the total pollen concentration obtained with the two differently oriented Button Samplers, the genus/family/group specific data demonstrated that the sampling efficiency is less sensitive to the wind direction for the aeroallergens of smaller aerodynamic sizes. Indeed, as Pinaceae pollen (65 μm) has a greater size and thus higher inertia, the wind direction has a visible effect on the sampling efficiency of these pollen (this finding corroborates well with the sampling efficiency data reported by Aizenberg et al. (2000a)). Our data show that if the target aeroallergen does not exceed about 24 μm aerodynamic diameter, performance of the Button Sampler does not show any significant dependence on the wind direction in outdoor environment.

3.3. Comparison of the total aeroallergen concentration data obtained with the Button Samplers and the Rotorod Sampler

As no statistically significant difference was observed earlier for fungal spore concentrations obtained with the two Button Samplers (SW and NE) (Table 1), the average aeroallergen concentration from the SW and NE samplers was calculated and used to compare with the Rotorod Sampler data set. The regression plot of the log-transformed data is presented in Fig. 2. In contrast to fungi, the pollen concentration data obtained with two Button Samplers showed statistically significant difference (Table 1). Therefore, the pollen concentration from the NE and SW Button Samplers were separately compared with the data recorded by the Rotorod Sampler. The regression plot between the log-transformed pollen data from the SW Button Sampler and the Rotorod Sampler is presented in Fig. 3a. Similar regression plot that shows the relationship between the data from the NE Button Sampler and the Rotorod Sampler is presented in Fig. 3b.

Fig. 2 demonstrates a positive correlation between fungal spore concentrations obtained with the two simultaneously operated Button Samplers and the Rotorod Sampler. Fig. 3a and b also demonstrate positive correlations between pollen data of the SW Button Sampler and the Rotorod Sampler as well as the NE Button Sampler and the Rotorod Sampler ($r = 0.79$ and 0.84, respectively). These conclusions may be used in future comparative studies of outdoor aeroallergens measured with the Button and the Rotorod samplers.

To quantify the differences in the aeroallergen concentrations obtained with the Button and the Rotorod samplers, a paired *t*-test was performed. As described above, the average data from two Button

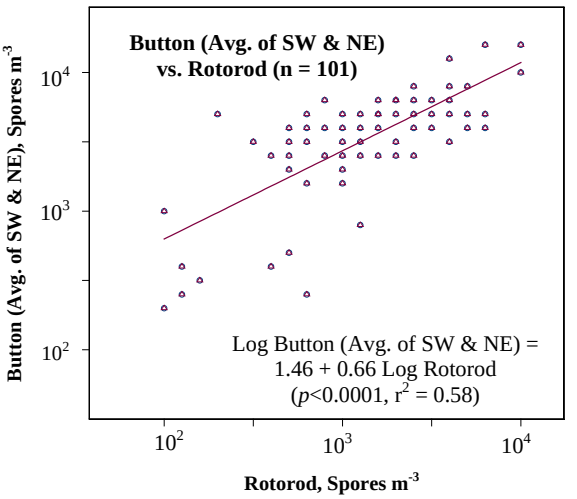
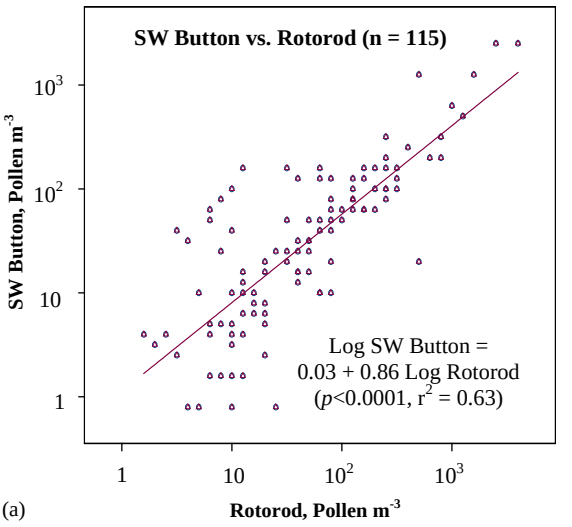


Fig. 2. Regression analysis plot for the log-transformed data on airborne concentrations of fungal spores (spores m^{-3}), measured by the two Button Samplers (average of SW and NE) and the Rotorod Sampler.

Table 2
Wilcoxon signed rank test analysis between the data obtained with two Button Samplers (SW and NE) measuring the concentrations for fungal spores (spores m^{-3}) and pollen grains (pollen m^{-3}) of four different sizes

Fungal and pollen genus/family/group (AD, μm)	SW Button (spores/pollen m^{-3})		NE Button (spores/pollen m^{-3})		*p*-Value (two-sided)
	Range	Median	Range	Median	
Spores $<5\,\mu m$ (2) ($n = 105$)	1–236	1419	1–446	1430	0.29
Alternaria (18) ($n = 69$)	2–107	80	1–192	80	0.71
Ambrosia (24) ($n = 35$)	11–1093	31	11–790	27	0.41
Pinaceae (65) ($n = 31$)	51–4735	24	27–5012	10	0.05



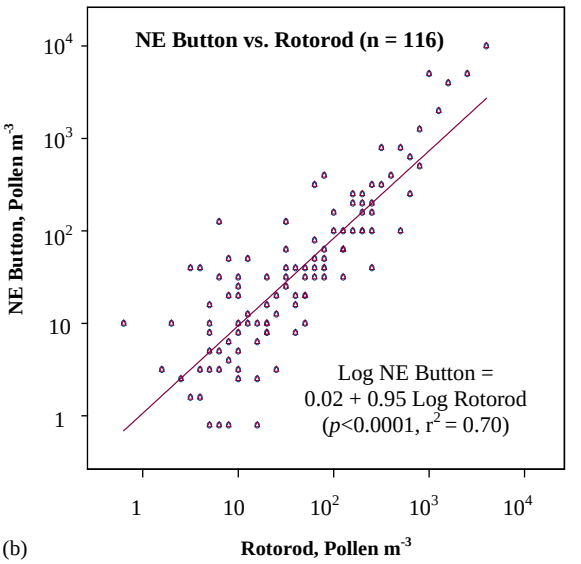


Fig. 3. Regression analysis plot for the log-transformed data on airborne concentrations of pollen grains (pollen m^{-3}) obtained by the SW Button Sampler and the Rotorod Sampler (a) and the NE Button Sampler and the Rotorod Sampler (b).

Samplers were used for fungal spores, while for pollen grains the two Button Sampler data (SW and NE) were each compared to the Rotorod Sampler data. The GM and the CVs of airborne concentrations are presented in Table 3.

For fungal spores, the average concentration obtained from the two simultaneously operated Button Samplers (3464 spores m^{-3}) was higher than the concentration recorded by the Rotorod Sampler (1495 spores m^{-3}). This difference was statistically significant ($p<0.001$). According to Di-Giovanni (1998), the sampling efficiency of the Rotorod Sampler increases as the particle size increases at a constant wind speed. Di-Giovanni referred to the experiment of Magill et al. (1968) that revealed 19% sampling efficiency of the Rotorod Sampler for *Clavatia gigantia* spores of 4–5 μm sizes, whereas the experiment of Aylor (1989) showed that this sampler had a sampling efficiency of 80% when collecting *Lycopodium* spores of 30 μm size. Frenz (1999) reported that the sampling efficiency of the Rotorod Sampler was <20% for the particles <10 μm, but it exceeded 80% when the particle size ranged between 20 and 60 μm. According to Frenz's study, the Rotorod Sampler's efficiency is lower for smaller fungal spores because the particles of relatively low inertia follow an airflow movement pattern around the collector's rods, which prevents them from impacting on the rotating rods. In contrast, larger particles are likely to deviate from the air streamlines and be subjected to the impaction. The Button Sampler does not utilize impaction, but filtration, which is proven to have high collection efficiency for wide range of bio-particle sizes (Aizenberg et al., 2000b). Unlike the Rotorod Sampler the Button Sampler is an inhalable sampler and thus, its inlet efficiency decreases with the particle size increase (Aizenberg et al., 1998, 2000a). Aizenberg et al. (2000a) reported that the Button Sampler's efficiency was 70% for particles of 7 μm size. Since about 75–90% of the fungal spores belonged to the group of 'spores <5 μm' and *Cladosporium* genera with small particle size (AD<5 μm), the Rotorod efficiency is considerably lower than that of the Button

Table 3
Paired *t*-test comparison between the data obtained with the Button Samplers and the Rotorod Sampler measuring the total concentrations for fungal spores (spores m^{-3}) and for pollen grains (pollen m^{-3})

Bio-particle type	Button Sampler		Rotorod Sampler		*p*-Value (two-sided)
	GM (spores/pollen m^{-3})	CV	GM (spores/pollen m^{-3})	CV	
Fungal spores[a] ($n = 101$)	3463	1.0	1495	1.3	<0.001[a]
Pollen grains[b] ($n = 115$)	33	7.2	37	4.4	0.31[b]
Pollen grains[c] ($n = 116$)	26	4.6	44	3.7	<0.001[c]

[a] Average of SW and NE Button vs. Rotorod.
[b] SW Button vs. Rotorod.
[c] NE Button vs. Rotorod.

Sampler, which explains the difference (3463 vs. 1495 spores m^{-3}) indicated in Table 3.

For pollen grains, both SW Button Sampler and NE Button Sampler showed lower GM of the concentration than the Rotorod Sampler (Table 3). While the difference between the NE Button Sampler set and the Rotorod Sampler set was statistically significant (26 vs. 44 pollen m^{-3}, $p<0.001$), the difference between the SW Button Sampler and the Rotorod Sampler sets was not statistically significant (33 vs. 37 pollen m^{-3}, $p=0.31$). Higher concentrations of pollen grains obtained by the Rotorod Sampler compared to the Button Sampler occurred because the Rotorod Sampler has the efficiency of about 80–100% for airborne pollen (Frenz, 1999), while the Button Sampler is an inhalable sampler with lower efficiencies for larger pollen grains, down to about 30% for 70 μm particles (Aizenberg et al., 2000a).

3.4. Comparison of the performance characteristics of the Button Samplers and the Rotorod Sampler for aeroallergens of different sizes

The ranges of aeroallergen concentrations and the median values of the distributions are presented in Table 4 for the group of 'spores <5 μm', *Alternaria, Ambrosia,* and Pinaceae. For Pinaceae pollen, the data from two Button Samplers were compared separately with the Rotorod Sampler because the data obtained with SW and NE-oriented Button Sampler were significantly different (Table 2). For other types of pollen and fungi, the average value was calculated for two simultaneously operated Button Samplers. For the group of 'spores <5 μm', a significantly higher concentration was observed by the Button Sampler compared to the Rotorod Sampler ($p<0.001$). However, for *Alternaria* spores and *Ambrosia* pollen no statistically significant differences were observed between the data obtained with the Button and Rotorod samplers ($p=0.26$ and 0.08, respectively). The concentration of *Alternaria* and other fungal species of relatively large particle sizes compared to the total fungal spore concentration was low. As described in Section 3.2, most of the samples showed predominantly the group of 'spores <5 μm' and *Cladosporium* (AD <5 μm). This fact explains why the total fungal spore concentration (Table 3, first row) demonstrated similar trends as the group of 'spores <5 μm': the Button Samplers showed significantly higher concentration than the Rotorod Sampler. It appears, that in the size range of *Alternaria* and *Ambrosia* (AD $\approx$ 18–24 μm), the Button Sampler and the Rotorod Sampler have comparable efficiencies. For Pinaceae pollen the situation is more complex. A higher concentration was recorded by the SW Button Sampler compared to the Rotorod Sampler, and the difference was statistically significant. However, the NE Button Sampler data showed no statistically significant difference with the Rotorod Sampler data. The total pollen concentrations showed somewhat different results: the Rotorod-recorded concentrations were similar to the SW Button-recorded ones, but exceeded the NE Button-recorded concentrations (Table 3). The difference between the Rotorod Sampler performances for Pinaceae count and the total pollen enumeration can be explained by a particular tendency of larger (e.g., Pinaceae) pollen grains to bounce off from the impaction surface of the Rotorod Sampler (Di-Giovanni, 1998).

3.5. Duplicate analysis of quality control samples

The average and SD of the ratios of the data obtained by two researchers analyzing the same samples are presented in Table 5. The quality control data confirm that the counts are consistent, and the subjective variability is reasonably low. The average ratio of the two counts ranged between 0.77 ± 0.22 and 1.00 ± 0.33. The highest standard deviation (0.55) was found for pollen collected with Button Samplers; 10 of the

Table 4
Wilcoxon signed rank test analysis between the data obtained with the Button Samplers and the Rotorod Sampler measuring the total concentrations for fungal spores (spores m^{-3}) and pollen grains (pollen m^{-3}) of four different sizes

Fungal and pollen genus/family/group (AD, μm)	Button Sampler (spores/pollen m^{-3})		Rotorod Sampler (spores/pollen m^{-3})		p-Value (two-sided)
	Range	Median	Range	Median	
Spores <5 μm[a] (2) ($n=102$)	43–4873	1449	0–196	0	<0.001[a]
Alternaria[a] (18) ($n=67$)	12–941	81	0–1028	100	0.26[a]
Ambrosia[a] (24) ($n=34$)	1–142	33	0–229	35	0.08[a]
Pinaceae[b] (65) ($n=33$)	1–236	15	0–123	8	0.03[b]
Pinaceae[c] (65) ($n=40$)	1–446	9	0–123	8	0.77[c]

[a] Average from SW and NE Button Samplers vs. Rotorod.
[b] SW Button vs. Rotorod.
[c] NE Button vs. Rotorod.

Table 5
Duplicate analysis of 7% of the samples obtained with the Button Samplers and the Rotorod Sampler

Sampler	Average ± SD of the ratio of the data obtained with duplicate analysis	
	Fungal spores	Pollen grains
Button Sampler ($n = 16$)	0.97 ± 0.29	0.88 ± 0.55
Rotorod Sampler ($n = 8$)	1.0 ± 0.33	0.77 ± 0.22

16 samples evaluated for quality control were taken on days when the pollen count was low (<10 pollen m^{-3}).

4. Conclusions

Overall, the data collected during a year-round outdoor field study indicate that the use of the Button Personal Inhalable Sampler is feasible and efficient for the measurement of the personal exposure to outdoor pollen and fungal spores. The collection efficiency of the Button Sampler has low sensitivity to the sampler orientation with respect to the wind. This property is especially notable for the airborne fungal spores. For the particles of larger size (e.g., Pinaceae pollen grains), the sampling efficiency of the Button Sampler are more dependent on its orientation. Use of two Button Samplers facing opposite to each other was found advantageous over the single sampler utilization while measuring pollen. The aerosol concentration data obtained with the Button Sampler is higher than that of the Rotorod Sampler for fungal spores. As long as the particle size is relatively small ($AD < 10\,\mu m$), the sampling efficiency of the Button Inhalable Aerosol Sampler is close to 100%, while the Rotorod's efficiency is lower due to the limited impaction of particles with lower inertia on a rod. For total pollen counts we found that either these samplers show the same performance, or the Button's readings are lower, reflecting that the sampling efficiency of the inhalable fraction is $<100\%$. Larger pollen grains, such as Pinaceae ($AD \approx 65\,\mu m$), tend to bounce off from the Rotorod collection rod resulting in lower counts from the Rotorod than from the Button.

Acknowledgements

The authors are thankful to Ms. Anne South for her help in data management and calculations. Thanks are also due to Mr. Shu-An Lee and Mrs. Hongxia Wang for their assistance in the sample collection. This work has been supported by the National Institute of Environmental Health Sciences (Grant No. R01 ES11170) as a part of the study on the "Diesel, Allergens and Gene Interaction and Child Atopy".

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