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Comparison of the Rotorod to other air samplers for the determination of *Ambrosia artemisiifolia* pollen concentrations conducted in the Environmental Exposure Unit

Matthew J. Heffer[1], Jodan D. Ratz[1], J. David. Miller[2,*], & James H. Day[1]
[1]*Division of Allergy and Immunology, Kingston General Hospital, Kingston, ON, Canada;* [2]*Department of Chemistry, Ottawa-Carleton Institute of Chemistry, College of Natural Sciences, Carleton University, 228 Steacie Building, Ottawa, Ontario, Canada K1S 5B6; (*Author for correspondence: Fax: +1-613-520-3749; E-mail: DavidMiller@pigeon.carleton.ca)*

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Abstract

The Environmental Exposure Unit (EEU) is a 924 m^3 facility (Kingston General Hospital, Ontario) in which uniform concentrations of various pollens in HEPA-filtered air at known rates of laminar airflow can be maintained. This facility provided a unique opportunity to compare several air samplers without the environmental variation inherent in outdoor comparisons. The purpose of this study was to conduct a quantitative comparison of pollen measurements using the Rotorod, Burkard™ Personal Volumetric Air Sampler, Air-O-Cell™ and a 37 mm open-faced filter cassette with a microporous filter in the EEU. Pollen samples were taken during clinical trials being conducted in the Unit. Raw pollen counts/m^3 obtained using the different methods were corrected using published particle collection efficiencies for the particle size ($\sim$20 μm) and airflow. Data were analyzed by ANOVA/Tukey HSD. No statistically significant differences were found between pollen concentrations determined by Rotorod, Air-O-Cell and filter cassette. Pollen levels determined by the Burkard were up to 2 times higher than the other sampling methods. Relative standard deviations were similar for the Rotorod, Burkard, and filter cassette and higher for the Air-O-Cell. This study demonstrated that, under our conditions, the Rotorod sampler provides consistent and reliable measurements of ragweed pollen concentrations.

Abbreviations: AOC – Air-O-Cell; Burkard – Burkard™ Personal Volumetric Air Sampler; EEU – Environmental Exposure Unit; filter cassette – 37-mm open-faced filter cassette with a microporous filter

1. Introduction

Allergic rhinitis is estimated to affect more than 10% of the North American population (Malone et al., 1997). Owing to the prevalence of allergic rhinitis and the relationship between pollen levels and symptoms (Frenz, 2001), there has been an increasing demand for accurate pollen counts. Information on the presence or absence of different pollen types and their respective concentrations can be used by physicians to help understand pollen trends thereby enabling better management of seasonal allergic rhinitis symptoms (Nelson and Solomon, 2003). Concentrations of airborne pollens are routinely measured using air samplers. In 1997, it was reported that

over 300 stations using Rotorod samplers were in use for routine aeroallergen monitoring in the USA (Frenz and Lince, 1997). Currently, pollen and spore data from the American Academy of Allergy, Astham & Immunology National Allergy Bureau comes from 85 stations, 17 of which use the Rotorod, while the remainder use Burkard-type samplers. In addition, there is a network of 30 Rotorod samplers in major cities in Canada (Aerobiology Research Laboratories, Ottawa).

A number of clinical studies that have been designed to compare anti-allergic medications used in the treatment of ragweed allergy have been reported by our unit (Day et al., 1997a, b; 2000; 2001; Day and Briscoe, 1999). These have been conducted in the Environmental Exposure Unit (EEU) at the Kingston General Hospital, Ontario (Canada), a facility designed to present predetermined levels of *Ambrosia artemisiifolia* (ragweed) and other pollens to subjects over the duration of a clinical study period independent of variables encountered in other study conditions. Over the past 10 years, thousands of Rotorod measurements have been made in the EEU because of the need to regulate and document exposure in each study. This prompted us to conduct a series of studies to compare the accuracy of the Rotorod sampler using the data obtained from rigorous side-by-side comparisons of different sampling methods.

This comparison was possible because, unlike the large temporal variations in pollen levels often measured in outdoor air (Watson, 1954), pollen concentration in the subject exposure area of the EEU is maintained within strict tolerances using measurements obtained from seven Rotorod samplers positioned throughout the seating area (Day and Briscoe, 1999). Additionally, and importantly, as sampling efficiency is strongly affected by air velocity across the sampling plane, the controlled air movement in the subject exposure area of the EEU facilitated this comparison. Air velocity is maintained at a constant speed in a single direction, in contrast to the varying wind direction and speed outdoors. Variable pollen concentrations and wind velocity make side-by-side comparisons of samplers conducted outdoors difficult to interpret. Other factors confounding sampler comparisons include: different sampler heights, surrounding structures, meteorological events, unequal sampling durations, adhesive application inconsistencies, as well as particle identification and counting errors. Variance in outdoor comparisons of air samplers are likely attributed to actual variability in pollen concentration as well as to differences in sampler performance.

The purpose of this report is to describe the results of experiments conducted in the controlled environment of the EEU, comparing the concentrations of *A. artemisiifolia* pollen as measured by Rotorod, Burkard™ Personal Volumetric Air Sampler (Burkard), Air-O-Cell™ (AOC and 37-mm open-faced filter cassette with a microporous filter (filter cassette).

2. Materials and methods

All measurements were conducted in the EEU while the facility was being used for clinical trials. Samplers were placed on a platform with the intake 1 m off the floor, corresponding to the breathing zone of a seated study participant. Air speed measured at the collection point was 0.6 ± 0.2 m/s, temperature was 23.4 ± 0.6 °C and relative humidity $55.5 \pm 5.5\%$. *A. artemisiifolia* pollen was purchased from Greer Laboratories (Lenoir, NC).

2.1. *Air samplers*

The Rotorod sampler (Model 85, Sampling Technologies Inc., Minnetonka, MN) was fitted with two plastic rods that were coated with silicone grease (Ted Brown Associates, Los Altos Hills, CA). To ensure that the preparation of rods was consistent (Gagnon and Comtois 1992), counts from the two rods of individual Rotorod samples were analyzed for variation in collection efficiency using a Pearson correlation and Wilcoxon sign test. The Rotorod sampled at 23.4 l/min per sampling rod (2 rods/sampler, 46.8 l/min). Before and after each use the sampler was calibrated with a stroboscopic tachometer to 2400 rpm ± 1% (Cole-Parmer phototachometer Model 08199-41, IL). The AOC sampling cassettes (Zefon International, St. Petersburg, FL) were connected to a Zefon high volume vacuum pump calibrated to 15 l/min. Room air was sampled with a 37-mm open-faced filter cassette with

a 0.45 μm polycarbonate filter (Zefon International, #FPC4537) at 10 l/min. The AOC and filter cassette were arranged with the intake opening facing into the direction of airflow. The Burkard Personal Volumetric Air Sampler (Rickmansworth, UK) was fitted with a standard microscope slide coated with silicone grease (as above) below the intake hole. Sampling rate was at 10 l/min operated with the intake orifice facing upward.

2.2. *Sample collection*

Each Burkard sample was collected for 1 min (10 l), 2 min (20 l) or 3 min (30 l). AOC samples were taken for 1 min (15 l) or 2 min (30 l). As each of these samples was collected, a Rotorod sampler operated for an equivalent period of time. The filter cassette was used with the same vacuum pump as the AOC calibrated at 15 l/min for a sampling period of 20 min (300 l). Five 1-min Rotorod samples were taken over this period at 0, 5, 10, 15 and 20 min to avoid overloading the sample rods.

2.3. *Pollen counting*

Ambrosia artemisiifolia pollen counting was done by light microscopy in duplicate for the Rotorod sampling rods, AOC, and Burkard slides; 100% of the sticky surfaces was counted in each case. Pollen on the filter membrane was determined by an adapted AIHA method (Dillon et al., 1996). A small volume (2 ml) of 0.1% Tween 80 solution was added to the cassette, which was then placed on a vortex mixer for 20 min. The resulting suspension was removed and pollen grains counted on a haemocytometer.

2.4. *Data analysis and statistics*

Pollen counts/m^3 were corrected using published particle collection efficiencies for 20 μm particles at the air velocity used (Di-Giovanni, 1998). The values chosen for correction calculations were based on empirical data where available. Rotorod: 68%, measured (Frenz, 2000; his Figure 1b, equation 2); Burkard 7-day recording spore trap: 90%, measured (Frenz, 1999; his Figure 2c, equation 11); AOC: 95%, calculated

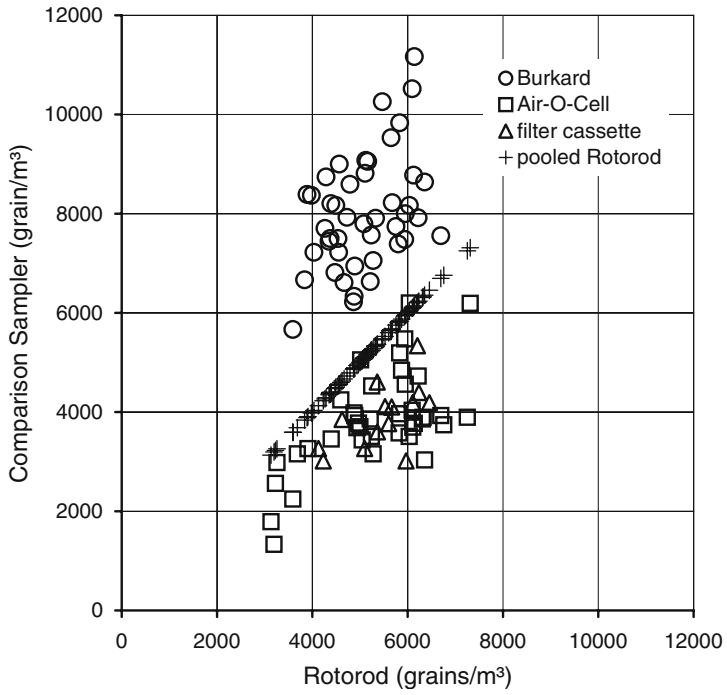

Figure 1. Scatter plot showing the corrected pollen counts for each sampler relative to the Rotorod. The pooled Rotorod data ($n = 85$) are shown as a reference line.

(Anon., 1998; p. 4); filter cassette: 65%, measured (Linden et al., 1997; their Figures 1 and 7). Data were analyzed with SPSS for Windows (ver 10.0.7) by one-way analysis of variance (ANOVA) and Tukey HSD *post hoc* test for multiple comparisons. A p value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. *Rotorod*

Pollen counts from each rod of the Rotorod sampler ($n=170$) showed a statistically significant correlation of $r=0.87$ ($p<0.001$) and the sign test confirmed the lack of a significant difference between the two rods of each Rotorod sample. Over the duration of the study, there was no statistical difference in pollen concentrations measured by the Rotorod sampler ($n=85$, 5144 ± 942 grains/m^3). Pollen data from the Rotorod sampler used in the comparison trials was correlated to the values obtained from the seven samplers placed throughout the chamber ($r=0.65$, $p<0.003$). This provides evidence of a uniform pollen distribution throughout the pollen exposure area, and, across the sampling plane.

Comparative data of the various samplers are shown in Figure 1. Absolute and corrected pollen counts are summarised in Table 1. The relative standard deviations of the uncorrected data were similar for the Rotorod, Burkard and filter cassette, and higher for the AOC (data not shown).

3.2. *Burkard Personal Volumetric Air Sampler (Burkard) vs. Rotorod*

The pollen grain concentrations for the Burkard and Rotorod over 44 trials are shown in Table 1. The mean ratio (corrected Burkard: corrected Rotorod) of recovery per m^3 for the trials was 1.6 ± 0.25. Sampled concentrations showed a significant correlation of $r=0.42$ ($n=44$, $p=0.004$; Figure 1). The different volumes sampled in this study (10, 20, and 30 l) had no statistically

Table 1. Absolute and corrected Ambrosia pollen grain concentrations collected by Rotorod, Burkard Personal Volumetric Air Sampler (Burkard), Air-O-Cell and 37-mm open-faced filter cassette with microporous filter (filter cassette)

	Rotorod	Corrected Rotorod[a]	Burkard	Corrected Burkard[a]
Rotorod: Burkard ($n=44$)				
Range (g/m^3)	2442–4553	3591–6696	5100–10050	5667–11167
Median (g/m^3)	3465	5095	7121	7912
Mean (g/m^3)	3457	5084	7207	8008
Standard deviation	529	778	1037	1152
	Rotorod	Corrected Rotorod[a]	Air-O-Cell	Corrected Air-O-Cell[a]
Rotorod: Air-O-Cell ($n=39$)				
Range (g/m^3)	2130–4973	3132–7313	1267–5889	1334–6199
Median (g/m^3)	3588	5276	3589	3778
Mean (g/m^3)	3619	5323	3648	3840
Standard deviation	766	1127	951	1001
	Rotorod	Corrected Rotorod[a]	filter cassette	Corrected filter cassette[a]
Rotorod: filter cassette ($n=14$)				
Range (g/m^3)	2807–4390	4128–6457	1959–3469	3014–5338
Median (g/m^3)	3783	5563	2585	3977
Mean (g/m^3)	3719	5469	2540	3907
Standard deviation	500	735	424	653

[a] Published particle collection efficiencies for particle size of 20 μm and airflow at 0.6 ± 0.2 m/s:
Rotorod, 68% (Frenz, 2000).
Burkard, 90% (Frenz, 1999).
Air-O-Cell, theoretical 95% (Anon., 1998).
Filter cassette, 65% (Linden et al., 1997).

significant effect on the calculated particle concentration.

3.3. *Air-O-Cell (AOC) vs. Rotorod*

The pollen grain concentrations for the AOC cassettes and Rotorod over 39 trials are shown in Table 1. The mean ratio (corrected AOC: corrected Rotorod) of recovery per m^3 for the trials was 0.73 ± 0.15. Sampled concentrations showed a significant correlation of $r = 0.65$ ($n = 39$, $p < 0.001$; Figure 1). The different volumes sampled (15, and 30 l) in this study had no statistically significant effect on the retained particle concentration.

3.4. *37-mm open-faced filter cassette with microporous filter (filter cassette) vs. Rotorod*

The pollen grain concentrations for the filter cassette and Rotorod for 14 trials are shown in Table 1. Only 14 trials were done due to the labour intensive enumeration procedure. The mean ratio (corrected filter cassette: corrected Rotorod) of recovery per m^3 for the trials was 0.72 ± 0.096. Sampled concentrations showed a significant correlation of $r = 0.59$, ($n = 14$, $p = 0.026$; Figure 1).

4. Discussion

The results from this study are unique in that the principal factors confounding similar comparisons conducted outdoors were controlled in the EEU. A crucial factor was the control of air movement. In the EEU, air speed was constant, airflow was in a single direction, and gusts were non-existent. Furthermore, pollen levels were predetermined, constant and reproducible, thereby preventing collection overloading and temporal variability. The samplers operated in parallel at the same height and 0.5 m apart, minimizing spatial differences. Only a single pollen type was present thereby eliminating counting errors due to species identification. All of these conditions assured consistent data.

The calculated and empirically determined collection efficiencies of the Rotorod for *Ambrosia* pollen have been reported as 85% (Frenz, 1999) and 68–71% (Ogden and Raynor, 1967; Di-Giovanni, 1998; Frenz, 1999), respectively. In the present study, the corrected Rotorod data result in values higher than corrected values for the filter cassette, a well characterized standard. This suggests that the higher, calculated collection efficiency for the Rotorod discussed by Di Giovanni (1998) of 85% is probably more correct at least under these conditions than the empirical values reported to date (68–71%; Di-Giovanni, 1998).

Although generally raw data from pollen samplers are reported, this is not typical of other measurements; all measuring devices have uncertainties. The failure to correct for varying sampling efficiency can result in large errors in the estimate for atmospheric pollen concentrations (Di-Giovanni, 1998). Because airspeed affects different samplers in different ways (e.g. Frenz, 2000), the present comparison has permitted us to consider sampler efficiency in our comparisons that is not possible in studies conducted outdoors.

Open-faced filter cassette sampling for particulates is a common particulate sampling method used in industrial hygiene. As a result there is a large amount of information on the performance of this method for many variables including particle size, airspeed and location of the cassette in relation to direction of air movement (e.g. Buchan et al., 1986; Linden et al., 1997). Our comparison of the filter cassette to the Rotorod at an air velocity of 0.6 m/s found that the mean ratio of corrected pollen concentrations per m^3 between these two samplers was 0.72 ± 0.096. Data from the filter cassette showed a significant correlation with the Rotorod data. There are few reports of the use of filter cassettes to count pollen, but it is recognized as quantitative for bacteria and fungi (Dillon et al., 1996).

Burkard Manufacturing, Inc. builds a variety of samplers based on the design by Hirst (Hirst, 1952). The majority of outdoor comparison data is obtained using the 'outdoor' Burkard Seven-day Volumetric Spore Trap, which has identical sampling characteristics, entry airspeed and sampling orifice design as the Personal Volumetric Air Sampler used in the present study. The two samplers differ in that the outdoor sampler head is mounted on a wind-vane while (like the indoor version of the 7-day spore trap), the personal sampler which has the intake orifice fixed in the

upright position. The effect of this difference, if any, would be that the difference is minimized at low airspeed (see Solomon et al., 1980) and unchanging wind direction, conditions provided in the EEU. Two studies have shown that the Burkard-style sampler gives values twice those of the Rotorod for particles of the size of *A. artemisiifolia* pollen (Solomon et al., 1980; Gagnon and Comtois, 1992). Since data were obtained from side-by-side comparisons conducted outdoors, they have not accounted for the effect of wind speed on sampling efficiency. Wind speed affects sampling efficiency of the two devices differently (Frenz, 1999; 2000). At a fixed airflow of 0.6 m/s, the ratio of pollen recovery of the Burkard sampler to the Rotorod was 1.6 ± 0.25. The pollen concentrations measured were significantly correlated to those obtained from the Rotorod. The different volumes collected with the Burkard had no effect on the resulting pollen concentration measurement. Although the particular Burkard sampler we tested (Personal Volumetric Air Sampler) was different in design from that of the Burkard samplers commonly used for pollen determinations outdoors, the recovery ratio we obtained with the Rotorod sampler was similar to the work of others with the outdoor Burkard (Solomon et al., 1980; Gagnon and Comtois, 1992). With respect to the differing sampler intake orifice orientation, a comparison of two samplers with the intake oriented to the wind direction and with the intake perpendicular to the wind found the relative collection efficiency was not related to air velocity (Portnoy et al., 2000). The aforementioned study tested two different volumetric samplers on a rooftop. Although the samplers differed with respect to wind orientation and sampling schedule, they displayed similar collection characteristics at all wind velocities for both pollen and spores. Based on the similarity of results obtained for the Burkard Personal Sampler and the outdoor unit, research by others on the effect of anisokinetic sampling, and the chosen evaluation conditions, it is reasonable to assume that our data from the Burkard Personal Volumetric Air Sampler can be extended to other Burkard models.

The AOC was designed to collect fungal spores that are much smaller than *A. artemisiifolia* pollen. Its performance is very similar to that of the Burkard for smaller particles (Linden et al., 1997; Aizenberg et al., 2000). In our evaluation, the relative ratio of pollen recovery obtained by this method compared to the Rotorod was 0.73 ± 0.15. Pollen concentrations measured by the AOC also showed a significant correlation with the Rotorod data. The different volumes sampled with the AOC had no effect on the resulting pollen concentration measurement. These data suggest that the AOC collection efficiency for particles the size of *A. artemisiifolia* pollen is lower than the manufacturer states (95%), but is still quite high.

These data do not demonstrate the superiority of one sampler over another, but rather indicate that caution should be used when comparing pollen levels obtained from different samplers. The application of this data to outdoor pollen concentrations is complicated by many factors. Correction of outdoor data requires knowledge of particle size, and wind conditions at the moment when the sample was recorded. It should also be noted that the AOC, Burkard, and filter cassette used in this study are not suitable for daily outdoor pollen collection.

5. Conclusions

Although the Rotorod has been used for determining pollen diversity and to estimate concentrations for over 40 years (Frenz and Lince, 1997), and is recognized to do so in a reproducible manner, further studies were required to confirm the quantitative value of the results obtained (Di-Giovanni, 1998; Coates et al., 2002). We have now demonstrated that the Rotorod measured pollen levels similar to the AOC and filter cassette measurement. In comparison, the Burkard over-estimated pollen concentrations by a ratio of 1.6:1. The Rotorod sampler provided consistent and reproducible measurements of ragweed pollen concentrations that are likely close to the absolute value.

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