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ORIGINAL PAPER

Preliminary studies on the effect of the Burkard alternate orifice on airborne fungal spore concentrations

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Abstract The Burkard 7-day spore trap with standard orifice is commonly used by researchers in sampling outdoor air. The alternate orifice is reported to have higher efficiency in collecting small airborne fungal spores; however, no previous studies compared Burkard samplers with different orifices. This study was conducted to study the effect of the alternate orifice on the concentration of airborne fungal spores. Air samples were collected from July to October 2005 with two Burkard spore traps, one had the standard orifice and the second had the alternate orifice. The two spore traps were located on the roof of a building (12 m height) at the University of Tulsa, Oklahoma. Burkard daily slides were analyzed for airborne spores by light microscopy. The data from the two samplers were statistically analyzed using *t*-tests. The results indicated that the alternate orifice had significantly higher concentrations of *Penicillium/Aspergillus*-type spores and basidiospores than the standard orifice. By contrast, the standard orifice had significantly higher concentrations of *Alternaria*, ascospores, and other spores than the alternate orifice. The alternate orifice can be used to increase the efficiency of trapping small spores, which can be underestimated by using the standard orifice. However, additional comparison in other months of the year is recommended.

Keywords Aerobiology · Airborne fungal spores · Alternate orifice · Burkard · Standard orifice

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1 Introduction

Various types of bioaerosol samplers are currently available. Selection of a sampler depends on sampler performance, expected concentration of bioaerosol, and method of analysis (Jensen et al. 1994); sampler performance has been extensively reviewed (Hinds 1982; Stetzenbach et al. 1992; Marple et al. 1993; Brockmann 1993; Baron and Willeke 1993; Grinshpun et al. 1994; Jensen et al. 1994; Lacey and Venette 1995; Buttner et al. 2002; Muilenberg 2003; Levetin 2004; Lacey and West 2006). Several laboratory and field studies compared various sampling methods; however, comparison of data from these studies is difficult because each study used different samplers, sampling times, volume of sampled air, methods of sample analysis, and types of bioaerosols (Solomon et al. 1980; Buttner and Stetzenbach 1993; Juozaitis et al. 1994; Mehta et al. 1996; Aizenberg et al. 2000; Portnoy et al. 2000; Lee et al. 2004; Grinshpun et al. 2005).

Impaction is the most commonly used method for bioaerosol collection. The Burkard 7-day spore trap is a slit impactor that is commonly used by researchers in

sampling the outdoor air to determine fungal spore and pollen levels (Muilenberg 2003; Levetin 2004; Lacey and West 2006). Previous studies have compared the efficiencies of a Burkard sampler with other air samplers. Solomon et al. (1980) compared the efficiencies of a Burkard spore trap, retracting Rotorod, and Rotroslide samplers in capturing pollen and fungal spores in an outdoor environment. The results indicated that recoveries from the spore trap were higher than the other two samplers for all particles, especially for small particles such as *Ganoderma* spores. They confirmed the advantage of suction traps for small particles. Buttner and Stetzenbach (1993) compared the efficiencies of an Andersen sampler and a Burkard spore trap in an indoor environment. Aizenberg et al. (2000) studied the difference between personal Burkard, Air-O-Cell, and Button samplers in collection of total airborne fungal spores. Portnoy et al. (2000) studied the performance of Burkard-24 h and Allergenco MK-3 volumetric collectors in outdoor air sampling. Grinshpun et al. (2005) indicated that the reduction in cut size of the air samplers increase their efficiencies for collecting small airborne fungal spores.

The orifice of a Burkard sampler is a single rectangular opening, which is 14 mm × 2 mm in size. The efficiency of the Burkard sampler is affected by the cut size of its orifice (d_{50}). The cut size (d_{50}) can be defined as the diameter of the particles of which 50% will be collected. The standard orifice of a Burkard sampler is the commonly used orifice, and its d_{50} is reported as 3.7 μm (Jensen et al. 1994; Buttner et al. 2002; Muilenberg 2003; Levetin 2004; Lacey and West 2006). However, in another study the d_{50} of the standard orifice was indicated to be 5.2 μm (Willeke and Macher 1999). Thus, Burkard samplers usually cannot efficiently collect particles with smaller diameter than the cut size (Buttner et al. 2002; Muilenberg 2003; Levetin 2004).

The alternate orifice is also called a high-efficiency orifice; its size is 14 mm × 2 mm at the intake, but it narrows down to 14 mm × 0.5 mm. The d_{50} of the alternate orifice is 2.17 μm. Therefore, it may capture spores of 2.17 μm in diameter and larger, and consequently can be used to increase the efficiency for trapping small spores, which may be underestimated by using the standard orifice (Jensen et al. 1994; Buttner et al. 2002; Levetin 2004). None of the previous studies compared 7-day Burkard samplers with different orifices.

The genera *Penicillium* and *Aspergillus* are causative agents of allergic conditions as well as possible toxin producers (Kendrick 2000). Also, some species of *Aspergillus* and *Penicillium* are capable of causing infections such as aspergillosis and penicillosis in immunosuppresed patients (Panackal et al. 2006; Hien et al. 2001). *Penicillium* and *Aspergillus* spores are significant components of the air of tropical and subtropical areas and are present as a minor constituent of the air spora of the temperate regions of Europe and North America as indicated in studies from different tropical and subtropical regions (Menezes et al. 2004; Rosas et al. 1993).

Penicillium/Aspergillus spores are small in size (2–6 μm); thus a greater concentration of these small airborne fungal spores may be captured by the alternate orifice of the Burkard sampler than when using the standard orifice. The main objective of this study is to compare the concentration of airborne fungal spores from the alternate orifice with those of standard orifice of the Burkard spore trap with the main focus on *Penicillium/Aspergillus* spores.

2 Materials and methods

2.1 Air sampling

Air samples were collected from 1st July to 31st October 2005 using two Burkard volumetric 7-day spore traps (Burkard Manufacturing Co., Rickmansworth, Hertfordshire, England). The spore traps were located on the roof of Oliphant Hall at the University of Tulsa (approximately 12 m height). The samplers were positioned 2 m apart in a northwest–southeast line (Fig. 1). The prevailing winds in Tulsa, Oklahoma are from the southwest so neither sampler would be blocking the other sampler under normal wind conditions. One spore trap was equipped with the standard orifice and the second had the alternate orifice. The same trap was used for the alternate orifice during the whole study, and the position of the traps was not changed during the study. The traps functioned continuously, drawing in air at a rate of 10 l/min. The flow rates of the two traps were checked weekly, and the orifices were cleaned weekly as well.

2.2 Samples preparation

The same method of sample preparation was used in both spore traps. A strip of Melenex tape was fixed on

the sampler drum and held in place with a small piece of double-sided sticky tape. Tape was coated with a thin film of Lubriseal stopcock grease (Thomas Scientific, Swedesboro NJ, USA). Airborne particles with sufficient inertia impacted on the tape beneath the orifice.

The sampler drums were changed weekly and the tapes cut into 48 mm segments representing the previous 7 days. Each tape segment was adhered to a microscope slide with 10% Gelvatol solution and allowed to dry. Cover slips were then applied with a few drops of glycerin jelly stained with basic fuchsin.

2.3 Sample analysis

The prepared slides were examined microscopically for fungal spore identification using an oil immersion lens (1,000× total magnification). Burkard daily slides were analyzed for some of the most common airborne fungal spores by light microscopy using the single longitudinal traverse method as previously described in Sterling et al. (1999). *Alternaria*, ascospores, basidiospores, *Cladosporium*, *Curvularia*, *Drechslera*, myxomycete spores, *Nigrospora*, other spores, *Penicillium/Aspergillus*, and smut spores were the most commonly counted spore types. Other spores included familiar spores such as *Torula*, *Spegazzinia*, *Periconia*, and *Cercospora*, which were infrequently seen, as well as unknown spore types. The concentration of each spore type, as well as the total concentration of all the fungal spores, were calculated and expressed as spores per cubic meter of air. Spore concentrations were log transformed for statistical analysis. Repeated-measures multivariate analysis of variance (MANOVA) and *t*-tests were used to compare the mean concentrations of each spore type as well as total spores concentration using Statistica 5.1 software (StatSoft, Inc., Tulsa, OK). Because multiple *t*-tests were performed to compare the means of various spore types, the Bonferroni correction was also calculated to adjust the alpha value downward to prevent falsely significant results.

3 Results

The results of repeated-measures MANOVA test showed that there was significant species effect ($F = 706.1702$; df = 12, 1,490; $P < 0.0001$), but no significant effect from the two orifices ($F = 3.3962$; df = 1, 1,490; $P > 0.05$). However, there was a significant interaction between sampler orifice and spore type ($F = 20.9597$; df = 12, 1,490; $P < 0.0001$). Thus, we applied the *t*-test to compare the concentrations of individual spore types. The results of the *t*-test showed that the alternate orifice yielded significantly higher concentrations of basidiospores, and *Penicillium/Aspergillus*-type spores (Table 1), whereas the standard orifice captured significantly higher concentrations of *Alternaria*, ascospores, and other spores (Table 1). Although there were differences in the relative abundance of various spores using the two orifices, the concentrations were significantly related based on correlations of the mean concentrations of the individual taxa ($r = 0.9912$, $P < 0.0001$).

Figure 2 shows the concentration of total airborne fungal spores from the alternate and the standard orifices throughout the 4 months. Higher concentrations of total airborne fungal spores were registered with the alternate orifice on 62 days of the total 123-day sampling period. The greatest difference in total spore concentrations from the two orifices was on 9th August with the concentration in the alternate orifice greater by 5,274 spores/m^3. The alternate orifice collected 1.7 times as many total spores than was collected by the standard orifice on that day. This difference was accounted for by the large difference in *Penicillium/Aspergillus* followed by basidiospores, ascospores, *Drechselra*, *Nigrospora*, *Cladosporium*, and smut spores. The mean concentrations of total airborne fungal spores were 9,023 spores/m^3 and 8,942 spores/m^3 for the alternate and standard orifices, respectively (Table 1).

The concentration of *Pencillium/Aspergillus* spores was higher using the alternate orifice on 100 of the 123 days sampling period (Fig. 3). The greatest difference between the two orifices for *Penicillium/Aspergillus* concentration was registered on 1st July when the concentration recorded by the alternate orifice was 1,769 spores/m^3 greater. On that day the alternate orifice registered approximately six times as many *Penicillium/Aspergillus* spores than was collected by the standard orifice. The mean *Penicillium/Aspergillus* spore concentration with the standard orifice during the 4 months was 470 spores/m^3, while that of the alternate orifice was 744 spores/m^3 (Table 1).

Table 1 Comparison of the 4-month mean concentration of various airborne fungal spores registered with Burkard spore traps with the standard and alternate orifices

Spores type	Mean concentration (spores/m^3)		Ratio of mean concentrations	T[a] values	P
	Standard orifice	Alternate orifice	Alternate/ standard		
Alternaria	244*	203	0.83	−3.7564	0.0001
Ascospore	1,587*	1,055	0.67	−11.5354	<0.0001
Basidiospores	1,144	1,390*	1.2	6.802	<0.0001
Cladosporium	4,671	4,905	1.1	1.9146	0.0289
Curvularia	44	40	0.91	0.6251	0.2665
Drecheslara	30	26	0.87	−0.449	0.3271
Epicoccum	44	39	0.89	0.0015	0.4994
Myxomycetes	34	36	1.1	1.205	0.1152
Nigrospora	29	26	0.90	0.8853	0.1889
Other spores	323*	201	0.62	−6.9382	<0.0001
Penicillium/Aspergillus	470	744*	1.60	8.6438	<0.0001
Pithomyces	22	19	0.86	−0.7085	0.2399
Smut spores	301	338	1.1	2.5690	0.0057
Total spores	8,942	9,023	1.01	0.01660	0.4934

[a] Log-transformed values used for statistical analysis

* $P < 0.00357$ based on Bonferroni correction

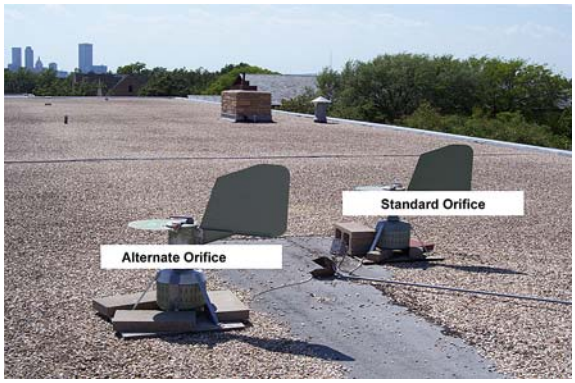

Fig. 1 The two Burkard samplers with two different orifices on the roof of Oliphant Hall, University of Tulsa

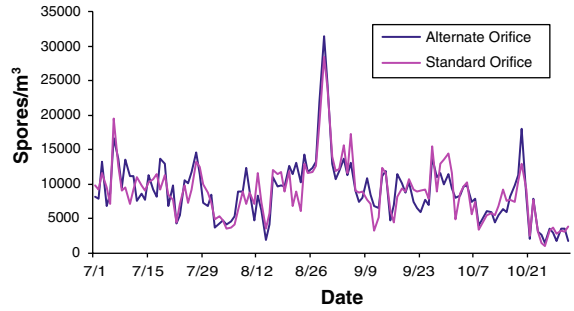

Fig. 2 Total airborne fungal spore concentrations registered with Burkard spore traps with two different orifices

The *Penicillium/Aspergillus* concentrations from the sampler with the alternate orifice showed a strong positive correlation with the concentrations registered by the standard orifice ($r = 0.5058$, $P < 0.0001$) (Fig. 4). The following regression model was determined from this 4-month comparison:

$$y = 0.5482x + 487,$$

where y represents the calculated concentration predicted for the alternate orifice, and x represents the measured concentration from the standard orifice.

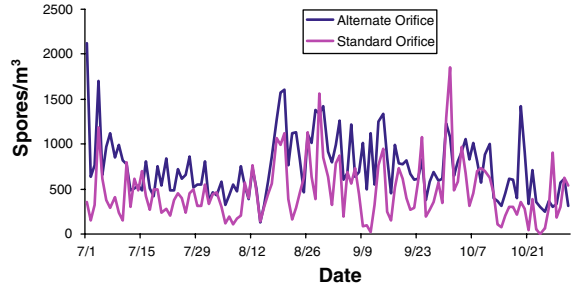

Fig. 3 *Penicillium/Aspergillus* spore concentrations registered with Burkard spore traps with two different orifices

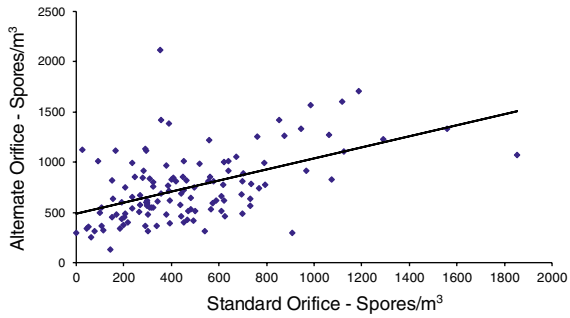

Fig. 4 Correlation of *Penicillium/Aspergillus* spore concentrations registered by two Burkard spore traps with standard and alternate orifices

4 Discussion

The results of this study demonstrate for the first time that the alternate orifice of the Burkard spore trap is more efficient than the standard orifice in collecting some types of the airborne fungal spores, especially small spores. By contrast, some spore types had lower concentrations registered by the alternate orifice. *Penicillium/Aspergillus*-type spores and basidiospores registered significantly higher concentrations with the alternate orifice, while *Alternaria*, ascospores, and other spores had significantly lower concentrations.

The orifice cut size can be considered as the diameter of the bioaerosol above which all particles are collected (Nevalainen et al. 1992). Therefore, the alternate orifice should efficiently collect spores of 2.17 μm and above. The standard orifice cut size is 3.7 μm (Buttner et al. 2002), and it should collect spores of diameter of 3.7 μm and above, so spores in the 2.17–3.7 μm range can be efficiently collected by the alternate orifice, but less efficiently collected by the standard orifice.

Based on the results of this preliminary study, it appears that the standard orifice fails to register all the airborne *Penicillium/Aspergillus* spores; nevertheless, the standard orifice is the one generally used by most investigators. The regression model described here $(y = 0.5482x + 487)$ may be useful for predicting the concentrations that would be obtained with an alternate orifice. However, further work is needed to validate the regression model throughout the year and for other locations.

The diameter of most *Penicillium* and *Aspergillus* spores is in the range 2–6 μm (Pitt 1991; Klich 2002). In another study we identified culturable xerophilic *Penicillium* and *Aspergillus* species in the Tulsa atmosphere at the ground (1.5 m) and rooftop levels throughout 2007 (unpublished data). We found that the most frequently isolated species and highest yearly average concentrations of *Penicillium* were *P. brevicompactum*, *P. citrinum*, and *P. implicatum*. Spores of these species are characterized by, a smooth surface, spherical to ellipsoidal shape, and having a small diameter of 2.2–3 μm (Pitt 1991). Also, *Aspergillus niger* was the most frequently isolated *Aspergillus* species in the yearly average concentration and frequency of isolation. The *A. niger* spores are globose with a diameter in the range 3.5–4.5 μm, but their surface is highly ornamented (Klich 2002).

A significantly higher mean basidiospore concentration was also registered by the Burkard spore trap with the alternate orifice. Basidiospores are often globose to elliptical in shape and single-celled; they vary greatly in size from small elliptical spores that are 1.5 × 3 μm to others that are 6 × 12 μm and larger (Pegler and Young 1971). It is possible that some of the smaller basidiospores were more efficiently captured by the sampler with the alternate orifice.

No previous studies have compared the standard and the alternate orifices of Burkard spore trap. The results of our study may confirm that the collection efficiency of the Burkard spore trap is affected by the size of its orifice: the smaller the orifice size, the higher the sampler collection efficiency for collecting some airborne fungal spores. This is in agreement with the results of previous studies that compared the sampling efficiencies of other air samplers (Aizenberg et al. 2000; Portnoy et al. 2000; Buttner and Stetzenbach 1993; Grinshpun et al. 2005). All these studies found that the efficiency of air samplers was dependent on the characteristics of the orifice as well as other factors. In our study the results of repeated-measures MANOVA test showed that there was a significant interaction of sampler orifice with spore type ($F = 20.9597$; df = 12, 1,490; $P < 0.0001$).

Grinshpun et al. (1994) studied the theoretical effect of orifice characteristics of several air samplers at different wind speeds in both indoor and outdoor air. They concluded that the collection efficiency of an air sampler orifice is affected by several physical and environmental factors. The physical factors

included shape, size, geometry, and orientation of the samplers orifice, and the density and size of the sampled airborne particles. Lacey and Venette (1995) and Nevalainen et al. (1992) also stated that the wind velocity can affect the collection efficiency of spore traps for different spore sizes. It is possible that the capture efficiencies of the standard and alternate orifice are different at different wind speeds.

The effect of meteorological conditions and seasonal variations on the concentration of airborne fungal spores has been well documented in many studies (Hasnain 1993; Hjelmroos 1993; Troutt and Levetin 2001; Millington and Corden 2005; Levetin and Dorsey 2006). Our study was conducted for only 4 months (July–October). Additional studies for longer sampling periods and in different seasons are necessary to confirm the results obtained in this study.

Previous studies have found differences in concentrations collected by the same types of air samplers (Solomon et al. 1980; Lembke et al. 1981; Hall 1992; Pedersen and Moseholm, 1993; Buttner and Stetzenbach 1993; Larsen-Purvis et al. 2004). Additional research using paired Burkard samplers with alternate and standard orifices is needed to determine whether the difference between the Burkard spore traps is due only to the difference in the size of their orifices, or if variation between samplers may contribute to the difference.

We noticed that the samples collected with the alternate orifice had more debris than the samples from the standard orifice. This may have caused some overloading of the greased surface and may have affected spore capture, especially of large spores. This study is a preliminary study and further research is recommended to explain the differences between the alternate and standard orifices in collecting airborne fungal spores.

5 Conclusions

The alternate orifice of Burkard spore trap collected significantly higher concentrations of *Penicillium/Aspergillus* spores and basidiospores than the standard orifice; however, the standard orifice had significantly higher concentrations of other spore types. The difference between the two orifices in collecting airborne fungal spores may be due to the effect of orifice size, sampled bioaerosol properties, meteorological factors, variation between samplers of the same type, or a combined effect of all or some of these factors. Thus, further research in different aspects is necessary to confirm the results of this preliminary study.

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