



An evaluation of two methods used for microscopic analysis of airborne fungal spore concentrations from the Burkard Spore Trap

Melinda Sterling, Christine Rogers & Estelle Levetin*

Faculty of Biological Science, University of Tulsa, Tulsa, OK, 74104, USA

(*author for correspondence, e-mail: biol_el@centum.utulsa.edu; fax: +1 918 6312762)

(Received 24 July 1997; accepted in final form 6 November 1998)

Key words: aerobiology, air spora, Burkard Spore Trap, counting methods, fungal spores

Abstract

The Burkard Volumetric Spore Trap is a common and efficient instrument used to collect outdoor air samples. In North America, two slide counting methods have been widely used by aerobiologists: the single longitudinal traverse method and the twelve transverse traverse method. The purpose of this study was to compare the two counting methods by assessing fungal spore concentrations of ascospores, basidiospores, smut teliospores, *Cladosporium*, *Alternaria*, *Epicoccum*, *Curvularia*, *Drechslera*, *Pithomyces*, other spores, and total spores at two metropolitan Tulsa, Oklahoma sites (Tulsa and Hectorville) during September 1996. Results showed that both methods were sensing parallel fluctuations in average daily spore concentration, although the twelve transverse traverse method usually resulted in higher concentrations. At the Tulsa site, the twelve transverse traverse method gave statistically higher concentrations than the single longitudinal traverse method except for *Epicoccum*, *Pithomyces*, smut teliospores, and other spores. At the Hectorville site, however, only *Cladosporium* and basidiospores showed that the twelve transverse traverse method was statistically higher than the single longitudinal traverse method. Comparison with concentrations obtained by counting the total slide surface of two slides indicated that neither method was equivalent to the total slide spore count, although the twelve transverse traverse method gave a lower absolute percent difference from the total slide surface concentration. While the twelve transverse traverse method gave slightly better approximations of the spore concentration, the increase in accuracy may not justify the extra effort required to analyze with this method.

1. Introduction

Sampling requires that a measured volume of air be collected that contains a representative fraction of the same kinds of particles as are present in the ambient air (Burge, 1992). Aerobiological sampling attempts to both identify and quantify allergenic or pathogenic particles in the ambient atmosphere and to aid in the diagnosis and treatment of allergy (Gutman and Bush, 1993; Lacey and Venette, 1995). Many sampling methods have been used since Pasteur first showed the presence of airborne microorganisms. Traditionally, an adhesive compound has been applied to a transparent surface or slide and the airborne particles

that adhere to the surface are counted microscopically (Gutman and Bush, 1993).

The development of the automatic volumetric spore trap (Hirst trap) increased our understanding of the air spora by demonstrating the abundance of previously ignored types such as ascospores and basidiospores (Lacey and Venette, 1995). Through the continual monitoring of airborne spores, this instrument allowed researchers to study the influence of meteorological factors on spore numbers and types, as well as examine the diurnal rhythms of spore dispersal (Lacey and Venette, 1995). The first version, which was created for research on the epidemiology of plant diseases, hay fever, and asthma, deposited particles onto a microscope slide that moved at 2 mm per hour

past an orifice (Hirst, 1952). Later versions (Burkard Manufacturing Company, Rickmansworth, UK) used an adhesive coated cellophane tape wrapped around a drum that also moved past an orifice at 2 mm per hour (Lacey and Venette, 1995; Muilenberg, 1995).

To determine the concentration of airborne spores, counts must be made of the particles adhering to the cellophane tape. The most accurate method would be to count the entire tape surface; however, this would be extremely time-consuming (Molina et al., 1996). In the normal day to day management of pollen and fungal spore counts from aerobiological samples, only a small proportion of the daily microscope slide is read. Otherwise, it is believed that the increased time spent reading a larger area of the slide will result in only a minimal increase in precision (Comtois et al., 1996). Several counting methods, which are based on examining a limited proportion of the tape, have been used to reduce time without reducing precision (Molina et al., 1996).

In North America, collections on slides from the Burkard Volumetric Spore Trap are commonly counted in two ways. The first method involves a subsampling unit, consisting of a single longitudinal traverse (single traverse) of the 48 mm tape surface, that upon conversion of the count to concentration represents the average concentration during the 24 hour period (Figure 1A). The second method uses subsampling units consisting of twelve transverse traverses (twelve traverse) at every 4 mm (2 hour intervals) along the length of the tape. With the twelve traverse method, counting is continued until no more spores are seen at the edge of the trace. Upon conversion of the counts to concentrations, each traverse or sweep represents the average concentration during one hour (Figure 1B). Because the trapping surface moves at 2 mm per hour past a 2 mm wide orifice, each point on the tape is exposed for one hour and the deposit represents a running hourly mean spore concentration. Time discrimination below one hour thus is not possible. The concentrations determined from the twelve traverses are then averaged to give an average daily concentration (Kapyla and Penttinen, 1981; Lacey and Venette, 1995). One of the main differences between the two methods is the time that it takes to read each slide. The twelve traverse counting method covers 3.5 times more area of the tape than the single traverse method and, therefore, generally takes 3.5 times as long to analyze.

There has been much debate on the efficiency and representiveness of each of the two methods, both

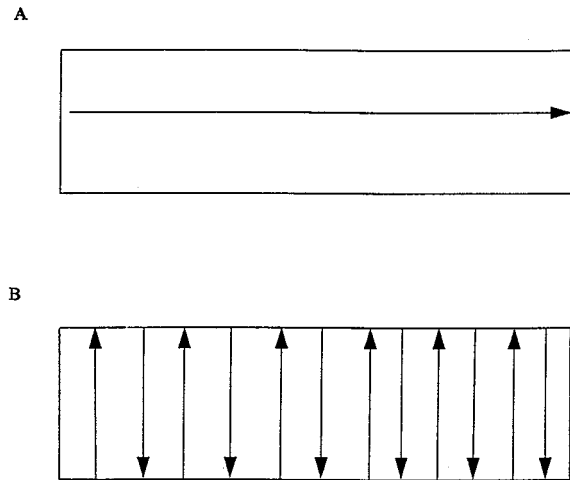


Figure 1. Locations of Microscope Traverses on Daily Slides using A. The Single Longitudinal Traverse Method, and B. The Twelve Transverse Traverse Method.

of which are widely used by aerobiologists. Little research, however, has been conducted to determine the most efficient and representative method, and most of the work that has been completed has focused on pollen. Kapyla and Penttinen (1981), after evaluating the two counting methods, concluded that twelve transverse traverses each two hours or at every 4 mm along the tape are sufficient to estimate the daily mean concentration of pollen at a magnification of $400\times$. The problem with applying this study to fungi is that a magnification of $1000\times$ is often necessary for accurate identification of most fungal spore types and, therefore, a much smaller area is being analyzed using both of these methods (Muilenberg, 1989; Burge, 1992). Fungi are not only smaller than pollen grains but are present in the air in concentrations considerably in excess of that of pollen grains (Salvaggio and Aukrust, 1981). For example, *Cladosporium* spores can outnumber pollen grains by a ratio of approximately a thousand to one.

Due to the differences between pollen and fungal spores, as well as the time required to analyze the slides using each method; the validity of these counting methods for the enumeration of fungi requires examination. The purpose of this research is to statistically compare the two common methods for analyzing Burkard slides and to determine the optimum counting procedure for fungal spores by analyzing data from two metropolitan Tulsa, Oklahoma sites.

2. Materials and methods

2.1. Sampling sites

Air samples for this study were obtained from the normal operation of Burkard Volumetric Spore Traps at two metropolitan Tulsa, Oklahoma sites. The largest portion of the material analyzed was collected from a Burkard Trap located on the roof of Oliphant Hall at The University of Tulsa in Tulsa. The sampler was set up approximately 12 m above the ground in an urban residential area. A second trap was located in a fenced enclosure in a pasture/range area in Hectorville. The sampler was positioned approximately 1.3 m above the ground on the crest of a small hill. Hectorville is a rural area located about 40 km southwest of Tulsa, Oklahoma.

2.2. Slide preparation and analysis

Airborne particles were deposited onto Melenex tape that was uniformly coated with a thin film of Lubriseal stopcock grease (Thomas Scientific, Swedesboro NJ, USA). After seven days of exposure, the tape was carefully removed from the drum and cut into daily (48 mm) segments. The tape was adhered to a microscope slide with a 10% Gelvatol solution and allowed to dry. Cover slips were then applied with a few drops of glycerin jelly stained with basic fuchsin. Finally, the slides were examined microscopically for fungal spore identification using an oil immersion lens (1000 \times magnification).

To determine the optimum counting method for fungal spores, concentrations of ascospores, basidiospores, smut teliospores, *Cladosporium*, *Alternaria*, *Epicoccum*, *Curvularia*, *Drechslera*, *Pithomyces*, an other category (all other fungal spores and unknowns), and total spores were determined microscopically using both the single longitudinal traverse and the twelve transverse traverse methods and statistically compared. In the twelve traverse method, the traverses were averaged to obtain the average daily concentration; while in the single traverse method the average daily concentration was determined directly. Concentrations were expressed as spores m⁻³ of air sampled. Air samples from the Tulsa site for the month of September 1996 and samples from Hectorville for the week of 4–10 September were examined with both methods.

The total slide surfaces of two slides from the Tulsa site (9 and 13 September 1996) were also examined to determine more accurate concentration totals for

each spore category. Eighty-two longitudinal traverses at 1000 \times magnification were required to microscopically examine the total slide surface of each slide.

2.3. Statistical analysis

All raw spore counts were entered into a Lotus spreadsheet, converted to concentrations of spores m⁻³, and imported into Statistica (5.0) software. Since the difference between the single and twelve traverse values cannot be assumed to be normally distributed, the nonparametric Wilcoxon Paired-Sample Test was used to determine statistically significant differences between these methods. Significant difference was assessed at a critical value of $p < 0.05$.

3. Results

3.1. Tulsa

Although the twelve traverse method usually measured higher fungal spore concentrations than the single traverse method during September 1996 in Tulsa, both methods generally showed similar average daily concentration fluctuations on the same days. *Cladosporium* illustrated this pattern beautifully (Figure 2). Although peaks occurred on different days for different taxa, similar patterns were observed with the two methods for most taxa.

For almost all fungal categories, however, there was at least one deviation in the average daily concentration between these two methods. For example, the single traverse method showed declines in total spore concentration on September 6 and 17 that the twelve traverse method did not show (Figure 3).

Epicoccum (Figure 4) and *Pithomyces* (Figure 5) showed parallel trends on some days but not on all days. For example, *Epicoccum* showed an increase with both methods on September 2 and a decrease on September 14–15 (Figure 4). There were increases, however, on September 17 and 25 observed with the twelve traverse method and increases on September 7 and 23 measured with the single traverse that were not detected with the other counting method.

The total spore concentrations (Figure 3) and the *Cladosporium* concentrations (Figure 2) as measured by both counting methods were very similar. *Cladosporium* was the most prevalent fungal spore type during September 1996 in the air samples from the Tulsa site. These spores, on most days during the month, comprised about one-half of the total air spora.

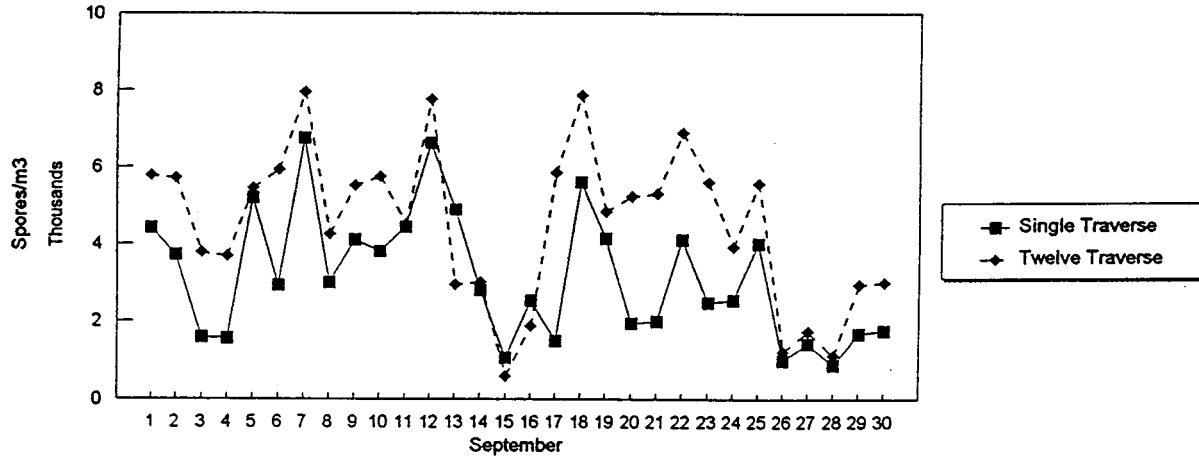


Figure 2. Average Daily Concentration of *Cladosporium* Spores in the Tulsa Atmosphere during September 1996.

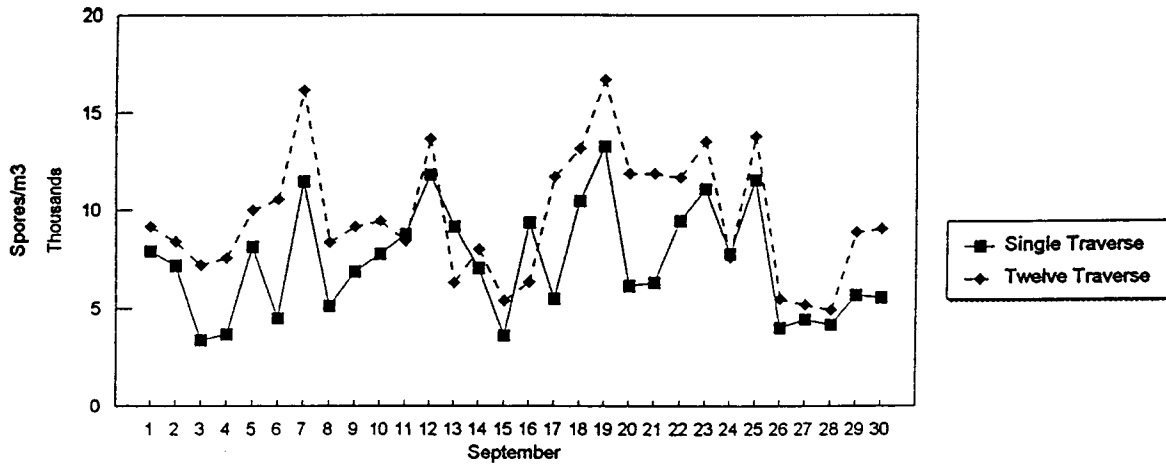


Figure 3. Average Daily Concentration of Total Spores in the Tulsa Atmosphere during September 1996.

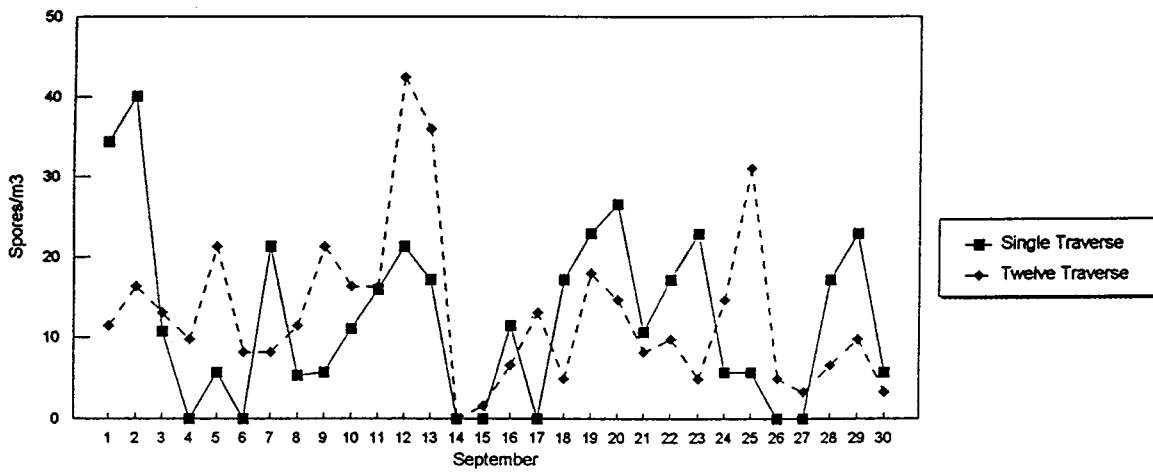


Figure 4. Average Daily Concentration of *Epicoccum* Spores in the Tulsa Atmosphere during September 1996.

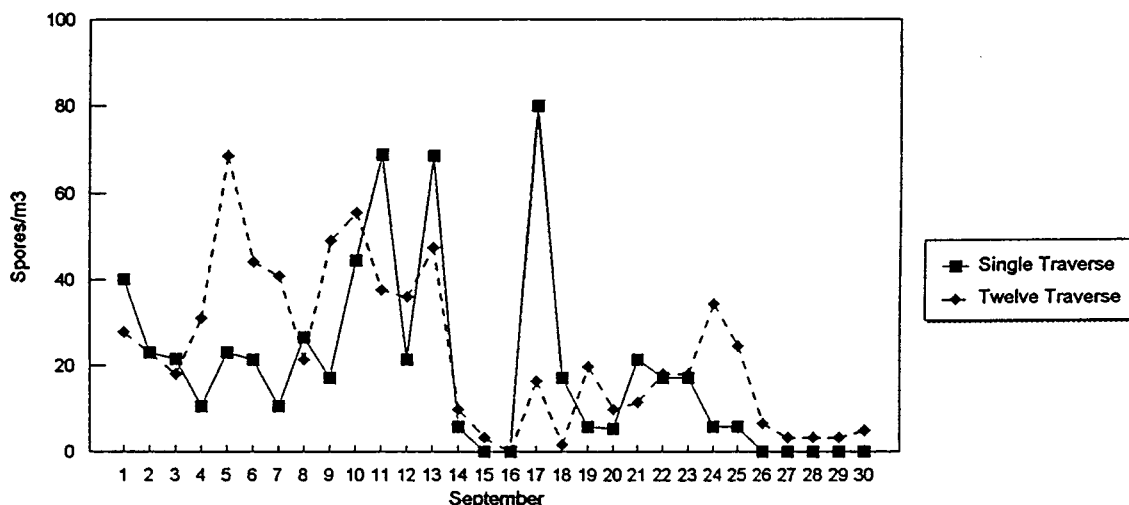


Figure 5. Average Daily Concentration of *Pithomyces* Spores in the Tulsa Atmosphere during September 1996.

On September 23 and 25, however, the concentration of total spores was mainly comprised of ascospores.

Mean concentrations for each fungal taxon/group were determined for the month using the data from both the single and twelve traverse methods (Table 1). Although the two methods showed similar fluctuations in the average daily concentrations, many of the mean concentrations determined by each method were significantly different. The mean concentrations using the twelve traverse method were significantly higher than the single traverse method for *Cladosporium*, *Alternaria*, *Curvularia*, *Drechslera*, ascospores, basidiospores, and total spores (Table 1).

3.2. Hectorville

As with the Tulsa samples, the twelve traverse method usually measured higher average daily concentrations than the single traverse method in the Hectorville samples. Both methods showed parallel concentration fluctuations for the same days. Again, *Cladosporium* illustrated this pattern (Figure 6). Parallel fluctuations could also be seen on different days for all taxa except *Epicoccum*. *Epicoccum* showed parallel concentrations on a few but not all of the seven days examined (Figure 7). As in Tulsa, total spore concentration showed curves similar to *Cladosporium* (Figure 6), which constituted about one-half of the total air spora.

Mean concentrations during 4–10 September 1996 for each fungal taxon were determined using the data from both counting methods (Table 2). Concentrations of total spores measured by the twelve traverse method were significantly higher than the single traverse

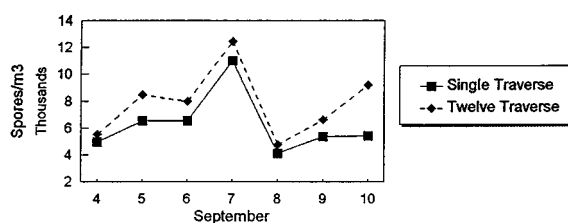


Figure 6. Average Daily Concentration of *Cladosporium* Spores in the Hectorville Atmosphere during 4–10 September 1996.

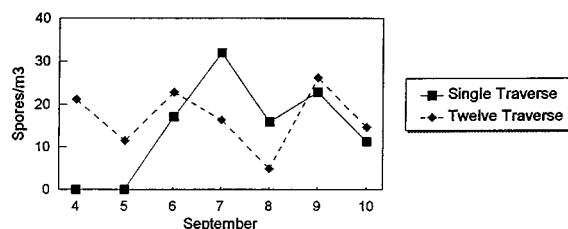


Figure 7. Average Daily Concentration of *Epicoccum* Spores in the Hectorville Atmosphere during 4–10 September 1996.

method. When individual taxa were examined, the mean concentrations measured by the twelve traverse method were significantly higher for only *Cladosporium* and basidiospores.

3.3. Total slide surface

The total slide surface of two slides (9 and 13 September 1996) was examined to more accurately establish the average daily concentration for all spore categories and to evaluate the accuracy of the two methods. The daily concentrations measured on 9

Table 1. Mean concentration of airborne spores during September 1996 in Tulsa, Oklahoma.

Fungal taxon or group	Method of analysis ^a	Mean conc. spores m ⁻³ ^b	Standard deviation	T ^c	p
<i>Cladosporium</i>	Single	3139	1653	34.00	0.000045*
	Twelve	4518	2007		
<i>Alternaria</i>	Single	160	115	125.00	0.027036*
	Twelve	208	148		
<i>Curvularia</i>	Single	43	31	98.00	0.005670*
	Twelve	68	51		
<i>Drechslera</i>	Single	42	34	100.50	0.006631*
	Twelve	64	48		
<i>Epicoccum</i>	Single	12	11	206.00	0.803620
	Twelve	13	10		
<i>Pithomyces</i>	Single	19	22	146.00	0.122100
	Twelve	23	18		
Smut teliospores	Single	282	199	170.00	0.198619
	Twelve	310	207		
Ascospores	Single	1523	1281	57.00	0.000307*
	Twelve	2155	1246		
Basidiospores	Single	1066	459	124.00	0.025644*
	Twelve	1298	702		
Other	Single	1077	605	201.00	0.517053
	Twelve	996	564		
Total spores	Single	7363	2797	40.00	0.000075*
	Twelve	9652	3193		

^a Single = Single Longitudinal Traverse; Twelve = Twelve Transverse Traverses at 4 mm intervals.

^b n = 30 days.

^c T = Wilcoxon Paired-Sample Test.

* Marked differences are significant at $p < 0.05$.

September 1996 by examining the total slide surface were generally in between the average daily concentrations determined by the two counting methods for most fungal taxa and total spores (Table 3 and Figure 8). The only exceptions were basidiospores and other spores. In contrast, both counting methods overestimated the daily concentrations for most taxa on 13 September 1996 when compared to the total slide surface count (Table 4 and Figure 8). The only exceptions were *Epicoccum*, smut teliospores, and basidiospores. Basidiospores were underestimated with both counting methods.

The absolute percent difference between each of the two counting methods and the total slide surface was calculated and compared for all fungal taxa on 9 September and 13 September 1996 (Tables 3 and 4).

For some spore types, the percent difference between the two counting methods and the total slide surface was fairly low; while for other spore types the percent difference was quite high. On both dates, the percent difference between the twelve traverse method and the total slide surface was small for smut teliospores with 1.8% and 8.5% (Tables 3 and 4). The smallest percent difference between the single traverse and the total surface was 1.1% for ascospores on 9 September 1996 (Table 3). By contrast, the percent difference between the single traverse method and the total surface was very large for *Pithomyces* and for other spores with 122.6% and 202.2% on 13 September 1996 (Table 4). The percent difference between the twelve traverse method and the total slide surface for ascospores was also quite high at 172.7% (Table 4).

Table 2. Mean concentration of airborne spores in Hectorville, Oklahoma during 4–10 September 1996.

Fungal taxon or group	Method of analysis ^a	Mean conc. spores m ^{-3b}	Standard deviation	T ^c	p
<i>Cladosporium</i>	Single	6281	2257	0.00	0.017966*
	Twelve	7862	2579		
<i>Alternaria</i>	Single	496	211	11.00	0.612093
	Twelve	537	256		
<i>Curvularia</i>	Single	131	49	7.00	0.236732
	Twelve	113	60		
<i>Drechslera</i>	Single	67	15	11.00	0.612093
	Twelve	76	33		
<i>Epicoccum</i>	Single	14	12	10.00	0.498967
	Twelve	17	7		
<i>Pithomyces</i>	Single	60	32	4.00	0.090979
	Twelve	74	32		
Smut teliospores	Single	366	124	10.00	0.498967
	Twelve	399	153		
Ascospores	Single	1830	905	11.00	0.612093
	Twelve	1849	521		
Basidiospores	Single	863	473	0.00	0.017966*
	Twelve	1574	1030		
Other	Single	1256	448	9.00	0.398031
	Twelve	1273	406		
Total Spores	Single	11366	4239	0.00	0.017966*
	Twelve	13773	4663		

^a Single = Single Longitudinal Traverse; Twelve = Twelve Transverse Traverses at 4mm intervals.

^b n = 7 days.

^c T = Wilcoxon Paired-Sample Test.

* Marked differences are significant at $p < 0.05$.

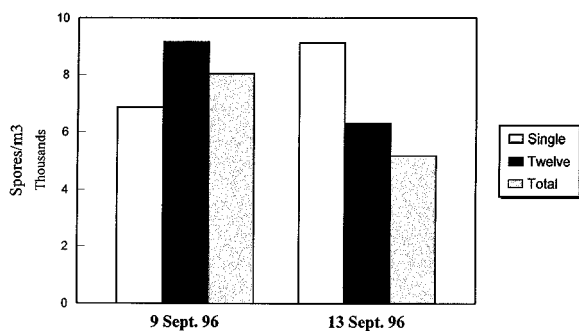


Figure 8. The 24-hour average concentration of total spores for 9 and 13 September 1996 presented by counting method: Single Longitudinal Traverse, Twelve Transverse Traverse, and Total Slide Surface.

The twelve traverse method had a lower percent difference for total spores when compared to the total slide surface on both dates (Tables 3 and 4). The twelve traverse method only showed a 13.8% difference on 9 September and 21.6% difference on 13 September 1996; while the single traverse had a 14.8% and 76.0% difference, respectively.

4. Discussion

At both the Tulsa and Hectorville sites, the average daily concentrations of airborne fungal spores measured by the twelve traverse method were usually higher than the single traverse method. Spore deposi-

Table 3. The average daily concentration of airborne spores in Tulsa, OK on 9 September 1996.

Fungal taxon or group	Single traverse		Twelve traverse		Total slide
	Concentration (spores m ⁻³)	% Difference from total	Concentration (spores m ⁻³)	% Difference from total	Concentration (spores m ⁻³)
<i>Cladosporium</i>	4100	(10.3)	5510	(20.5)	4573
<i>Alternaria</i>	206	(29.5)	418	(43.2)	292
<i>Curvularia</i>	69	(10.4)	82	(6.5)	77
<i>Drechslera</i>	40	(42.0)	83	(20.3)	69
<i>Epicoccum</i>	6	(66.7)	21	(16.7)	18
<i>Pithomyces</i>	17	(46.9)	49	(53.1)	32
Smut Teliospores	194	(10.3)	225	(1.8)	221
Ascospores	749	(1.1)	1124	(48.5)	757
Basidiospores	675	(55.4)	792	(47.6)	1512
Other	807	(63.0)	846	(70.9)	492
Total spores	6857	(14.8)	9152	(13.8)	8045

Table 4. The average daily concentration of airborne spores in Tulsa, OK on 13 September 1996.

Fungal taxon or group	Single traverse		Twelve traverse		Total slide
	Concentration (spores m ⁻³)	% Difference from total	Concentration (spores m ⁻³)	% Difference from total	Concentration (spores m ⁻³)
<i>Cladosporium</i>	4873	(99.3)	2946	(20.5)	2445
<i>Alternaria</i>	252	(24.8)	270	(33.7)	202
<i>Curvularia</i>	80	(90.5)	69	(64.3)	42
<i>Drechslera</i>	57	(32.6)	54	(25.6)	43
<i>Epicoccum</i>	17	(32.0)	36	(44.0)	25
<i>Pithomyces</i>	69	(122.6)	47	(51.6)	31
Smut teliospores	486	(65.9)	268	(8.5)	293
Ascospores	652	(39.9)	1271	(172.7)	466
Basidiospores	829	(20.1)	595	(42.7)	1038
Other	1813	(202.2)	750	(25.0)	600
Total spores	9127	(76.0)	6306	(21.6)	5185

tion is not uniform along the width or length of the trace due to differences in spore size and changes in external wind speed (Kapyła and Penttinen, 1981; Lacey and Venette, 1995). Often there are two zones of dense deposit along the long axis that result from uneven particle distribution. These two zones of dense deposit are counted with the twelve traverse method but not with the single traverse method. Consequently, the mean of the twelve traverse method is often greater than the daily average determined with the single traverse method. The difference may be less, however, if the traverse was moved off the center line and into one of the zones of higher deposition.

Both the twelve and single traverse methods measured similar fluctuations in the average daily concentrations of airborne spores indicating that both methods were sensing the daily variation of fungal spores. The few differences observed, i.e. when the twelve or single traverse method yielded an increase/decrease in concentration that the other method did not show, may be partly attributed to the uneven particle distribution of the spores across the trace. For example, the random occurrence of a cluster of a single type of fungal spore, regardless of the method, will increase the concentration accordingly.

At the Tulsa site, the average daily concentrations measured by the twelve traverse method were significantly higher than the single traverse method for the

fungal taxa/groups examined except for *Epicoccum*, *Pithomyces*, smut teliospores, and other spores. The significant difference between the two methods in abundant genera, such as *Cladosporium*, was due to the twelve traverse method registering higher concentrations than the single traverse method nearly all of the time. *Epicoccum* and *Pithomyces*, however, had the two lowest daily concentration means of all the spore types. Kapyla and Penttinen (1981) showed that the efficiency of estimating the mean concentration decreases with the fewer number of particles counted due to increased variance. The smut teliospore group and the other group contained several to many species. The results may have been different if individual genera within each group had been counted rather than placed in one category.

At the Hectorville site, only *Cladosporium*, basidiospores, and total spores showed that the twelve traverse method was significantly higher than the single traverse method. It was not surprising that total spores were significantly higher, because *Cladosporium* concentrations were significantly different and contributed to approximately one-half of the total spore concentration. Although the means for all other fungal taxa/groups were not significantly different, the means for the twelve traverse method were usually higher than those for the single traverse method. The lack of statistical difference between the counting methods at Hectorville could have occurred because only seven days were examined at Hectorville; while thirty days were examined at the Tulsa site.

The total slide surface concentrations were closer to the single traverse concentration for some taxa but closer to the twelve traverse concentration for other taxa. For smut teliospores, the twelve traverse concentration was much closer to the total slide surface concentration than the single traverse concentration with absolute percent differences of 1.8% on 9 September 1996 and 8.5% on 13 September 1996. For the ascospores, the single traverse concentration was much closer to the total slide surface concentration than the twelve traverse concentration. The percent differences were only 1.1% on 9 September 1996 and 39.9% on 13 September 1996. It is unclear, however, why the ascospores were better estimated with the single traverse method and the smut teliospores were better estimated with the twelve traverse method. It is also not clear why basidiospores were underestimated dramatically with each of the counting methods. Overall, the twelve traverse concentration was closer to the total slide surface concentration than the single

traverse concentration for total spores with 13.9% difference on 9 September 1996 and 21.6% difference on 13 September 1996.

Although only the total slide surface of two slides was examined due to time constraints (82 longitudinal traverses per slide), inferences can be made. Kapyla and Penttinen (1981), when comparing the twelve traverse method and the single traverse method, showed that for *Artemisia* pollen the twelve traverse method was needed for a reliable estimation of the daily mean but for *Betula* pollen very few traverses are needed. Twelve traverses thus seemed to be enough in all cases when compared to the total slide surface. In the current study, the daily concentration value obtained from the total slide surface was in between the two counting methods for 9 September 1996. On 13 September 1996, the daily concentration measured by the total slide surface was less than both methods. Although the twelve traverse method was closer than the single traverse method, both methods overestimated the actual concentration.

The differences between the current results and Kapyla and Penttinen's (1981) study may be attributed to the differences between pollen and spores: relative size, atmospheric concentrations of spores, and the magnification needed for identification and enumeration. Pollen grains are larger and less numerous in the atmosphere than fungal spores. A magnification of 1000 \times is often required for many fungal spores while 400 \times is sufficient for pollen grains.

The difference may also be attributed to the number of total slide surfaces that were examined in Kapyla and Penttinen's (1981) study and in the current study. Kapyla and Penttinen (1981) examined the total slide surface of eight slides (two for *Betula* and six for *Artemisia*). In the current study, however, the total slide surface of only two slides was examined for the ten taxa. In Kapyla and Penttinen's (1981) study, the average daily concentrations as measured by the twelve traverse method were almost exactly equal to the total slide surface concentrations. The smallest difference in concentration was 0.3 grains m^{-3} and the largest difference was 5 grains m^{-3} . In the current study, however, the results were much more variable. The smallest difference in average daily concentration measured by the twelve traverse method was 3 spores m^{-3} and the largest difference was 1107 spores m^{-3} .

Kapyla and Penttinen (1981) also recommended that if using the longitudinal method for pollen counts, several traverses should be studied due to the irreg-

ularities in the distribution of pollen grains across the tape. Molina et al. (1996) found that four single longitudinal traverses were not equivalent to (less than) the total pollen counts obtained from examining 18 longitudinal traverses. The results from the single traverse method in this study indicate that the single traverse was consistently lower than the twelve traverse method but not always statistically lower.

Kapyla and Penttinen (1981) found that there was always a general trend of decreasing estimation error with increasing the percentage of the slide read. The twelve traverse method analyzes 3.5 times as much of the slide as the single traverse method. It could be assumed that the twelve traverse method would, therefore, have a lower concentration error estimation and it would be more accurate than the single traverse method. The twelve traverse method had a lower absolute percent difference than the single traverse method when compared to the total slide surface for total spores on 9 and 13 September. A larger number of the total slide examinations, however, are required to establish whether the twelve traverse method would consistently give a better estimate.

This study focussed on counting error, however, this is not the only source of error in aerobiological sampling. Other types of sampling error include the use of different samplers, different counters of the microscope slides, and identification errors. Solomon et al. (1980), when using more than one sampler of the same type and analyzing a large number of samples, uncovered quite small but consistent differences in collection by individual samplers. According to Burge (1995), there are few people capable of identifying even a small portion of the ambient fungus aerosol. Identification error, therefore, has the potential to be quite large.

In conclusion, both methods examined in this study were sensing parallel fluctuations in average daily fungal spore concentrations, although the twelve traverse method usually resulted in higher concentrations. Comparison of the total slide surface of two slides indicated that neither method was equivalent to the actual spore count. While the twelve transverse method gave slightly better approximations of total slide spore concentration, many more days of comparison are required to conclusively establish the

higher accuracy of this method. Even though the twelve traverse method is more accurate for some spore types, the increase in accuracy will not always be justified due to the extra effort required to count particles with this method.

Acknowledgements

The authors would like to acknowledge Dr. Paul Buck for his help with the manuscript, and Crystal Icenhour for changing the samplers. This research was made possible, in part, by an NSF-EPSCoR grant to enhance the Oklahoma Mesonet (Project Number EPS9550478) and by the University of Tulsa Research Office.

References

- Burge H.: 1992, Monitoring for airborne allergens. *Ann. Allergy* **69**, 9–18.
- Burge H.: 1995, Bioaerosol Investigations. In: H. Burge (ed), *Bioaerosols*. Lewis Publishers, Boca Raton, FL, pp. 1–23.
- Comtois P., Alcazar P. and Neron D.: 1996, (Abstr.) Pollen count statistics and its relevance to precision. Compostela Aerobiology. European Symposium on Aerobiology.
- Gutman A. and Bush R.: 1993, Allergens and other factors important in atopic disease. In: R. Patterson, C.L. Grammer, P.A. Greenberger and G.R. Zeiss, *Allergic Diseases: Diagnosis and Management*, 4th edition. J. B. Lippincott Company, Philadelphia, pp. 93–158.
- Hirst J.M.: 1952, An automatic volumetric spore trap. *Ann. Appl. Biol.* **39**, 257–265.
- Kapyla M. and Penttinen A.: 1981, An evaluation of the microscopic counting methods of the tape in Hirst-Burkard pollen and spore trap. *Grana* **20**, 131–141.
- Lacey J. and Venette J.: 1995, Outdoor air sampling techniques. In: C.S. Cox and C.M. Wathes (eds), *Bioaerosol Handbook*. CRC Lewis Publications, Boca Raton, FL, pp. 407–471.
- Molina R.T., Rodriguez A.M. and Palacios I.S.: 1996, Sampling in aerobiology. Differences between traverses along the length of the slide in Hirst spore traps. *Aerobiologia* **12**, 161–166.
- Muilenberg M.: 1989, Aeroallergen assessment by microscopy and culture. *Immunol. Allergy Clin. N. Am.* **9**(2), 245–268.
- Muilenberg M.: 1995, The Outdoor Aerosol. In: H. Burge (ed), *Bioaerosols*. Lewis Publishers, Boca Raton, FL, pp. 163–204.
- Salvaggio J. and Aukrust L.: 1981, Mold-induced asthma. *J. Allergy Clin. Immunol.* **68**(5), 327–346.
- Solomon W.R., Burge H.A., Boise J.R. and Becker M.: 1980, Comparative particle recoveries by the Retracting Rotorod, Rotoslide and Burkard Spore Trap sampling in a compact array. *Int. J. Biometeor.* **24**(2), 107–116.