Effect of sampling height on the concentration of airborne fungal spores

Abeer Khattab, PhD, and Estelle Levetin, PhD

Background: Spores of many fungal species have been documented as important aeroallergens. Airborne fungal spores are commonly collected from the outdoor air at the rooftop level of high buildings; however, human exposure usually occurs nearer to the ground. It is necessary to estimate the concentration of airborne fungal spores at the human breathing level to evaluate the actual human exposure to outdoor aeroallergens.

Objective: To compare the concentration of airborne fungal spores at human respiration level (1.5 m above the ground) and at roof level (12 m height).

Methods: Air samples were collected using 2 Burkard volumetric 7-day recording spore traps from July 1 to October 31, 2005. One sampler was located on the roof of a building at the University of Tulsa at 12 m above ground, and the second sampler was placed in the courtyard of the building at 1.5 m. Burkard slides were analyzed for fungal spores by light microscopy at a magnification of 1,000, and the results were statistically analyzed to compare the concentration of airborne fungal spores at the 2 levels.

Results: The ground sampler had significantly higher concentration of basidiospores, *Penicillium/Aspergillus*-type spores, and smut spores than the roof sampler. By contrast, the rooftop sampler registered significantly higher concentration of *Alternaria*, ascospores, and other spores.

Conclusions: Ground level had significantly higher concentration of some important fungal aeroallergens but lower concentrations of others, suggesting that sampling height is one of the many variables that influence bioaerosol levels.

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INTRODUCTION

Fungal spores represent a major component of the bioaerosols in outdoor and indoor environments throughout the world.[1,2] Spores of many fungal species have been documented as important aeroallergens.[1,3–5] *Alternaria*, *Aspergillus*, basidiospores, *Cladosporium*, *Curvularia*, *Drechslera*, *Epicoccum*, *Fusarium*, *Penicillium*, smut spores, and *Trichoderma* are some of the common allergenic fungi.[5] Exposure to some fungal spores can also trigger infectious diseases for immune compromised persons.[6,7]

Human exposure to aeroallergens usually occurs close to ground level at approximately 1.5 m. By contrast, airborne fungal spores and pollen are commonly collected from the outdoor air by fixed spore trap samplers on the roof of high buildings (often 10 to 30 m or more above ground).[8,9] Sampling airborne fungal spores at these heights may underestimate spore concentrations of some important aeroallergens.[9] Thus, it may be necessary to have other sites at the ground or human respiration level (1.5 m above the ground) to detect the concentrations of pollen and fungal spores at this level.[9,10]

The atmosphere is characterized as being layered. It is known that atmospheric properties, such as barometric pressure, density of the air, and temperature decrease with increasing height above the ground level. These changes may affect the bioaerosols. The troposphere region is the lower layer of the atmosphere that extends from the ground up to a height of approximately 10 km. The temperature decreases as the height increases in the troposphere, and theoretically, in stable conditions near the ground level, spore concentrations decrease logarithmically with increasing height.[11]

Selection of sampler location and height is important in studying bioaerosols. There is a general agreement for using rooftops for sampling outdoor bioaerosols, because the registration is considered to be representative of bioaerosols in the region and away from the effect of local sources and possible sources of air pollution.[8] In addition, it is high enough to avoid vandalism and bothering neighbors with sampler noise. However, a standard sampling height has not been documented.[8,10] On the other hand, the issue of the suitable height of air samplers has been studied.[9,10,12–17] Differences in pollen concentrations at different heights have been observed. Some studies showed that the sampling height affected pollen count, and high concentrations of some taxa were found at lower sampling heights, depending on the source and the size of pollen grains.[10,13–17]

Several researchers interested in studying fungal aerollergens in the indoor or outdoor air collected samples using various sampling methods from ground level to human breathing height.[18–32] However, few studies have compared the concentration of outdoor airborne fungal spores at different heights.[9,10,12,17]

Affiliations: Biological Science Department, The University of Tulsa, Tulsa, Oklahoma.

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Previous studies (Khattab and Levetin, unpublished data, 2005) compared outdoor airborne fungal spore concentrations of some taxa in Tulsa and other sampling sites in northeast Oklahoma. The sampling heights were rooftop level (height of 12 m) in Tulsa and human breathing level (height of 1.5 m) in open fields in the other cities. Significantly higher concentrations were found of some spore types at 1.5 m than rooftop level; however, the samplers were not at the same site. The main objective of the current study was to compare the concentration of outdoor airborne fungal spores at the human breathing level with those from the rooftop level at the same location.

METHODS

Air Sampling

Air samples were collected from July 1 through October 31, 2005, using 2 Burkard volumetric 7-day spore traps (Burkard Manufacturing Company, Rickmansworth, Hertfordshire, England) with the standard orifice. One spore trap was located on the roof of a building at the University of Tulsa (approximately 12 m high), and the second spore trap was placed in the courtyard of the same building at height of 1.5 m. The difference in height between the 2 spore traps was approximately 10.5 m. The courtyard of the building is 12.5 $\times$ 26 m and is enclosed by the building. On the east and south sides the building is 3 stories tall, whereas on the north and west sides it is a single story. The vegetation in the courtyard consists of trees, shrubs, and herbaceous plants native to Oklahoma. In addition, a small artificial stream runs through the enclosed area. The sampler was placed near the center of the courtyard.

Sample Preparation

The same method of sample preparation was used for both spore traps. A strip of tape was fixed on the sampler drum and held in place with a small piece of double-stick tape. The tape was coated with a thin film of stopcock grease (Lubriseal; Thomas Scientific, Swedesboro, New Jersey). Airborne particles with sufficient inertia were impacted on tape beneath the orifice. The impaction surface moved past the orifice at 2 mm/h.

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 a 10% Gelvatol solution and allowed to dry. Coverslips were then applied with a few drops of glycerin jelly stained with basic fuchsin.

Sample Analysis

The prepared slides were examined microscopically for fungal spore identification using an oil immersion lens ($\times$1,000 magnification). Burkard daily slides were analyzed for some of the most common and important airborne fungal spores by light microscopy using the single longitudinal traverse method.[33] *Alternaria,* ascospores, basidiospores, *Cladosporium, Curvularia, Drechslera, Epicoccum,* myxomycetes, *Nigrospora,* other spores, *Penicillium/Aspergillus,* and smuts were the most commonly counted spore types. The category "other spores" contained known but infrequently seen spores, including *Torula, Cercospora, Spegazzinia, Periconia,* and *Fusarium,* 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. Statistica 5.1 (StatSoft Inc, Tulsa, Oklahoma) software was used to determine the relationship between the rooftop and the ground levels for airborne fungal spore concentrations using repeated-measures multivariate analysis of variance (MANOVA) and *t* tests. Bonferroni correction was applied to determine the statistical significance of *t* tests.

RESULTS

Some fungal spore types were recorded with higher concentrations at breathing level, and other types were higher at the rooftop level. The results of repeated-measures MANOVA test showed that there was significant effect in species-species comparison ($F_{12,1586} = 603.54$; $P < .001$) but no significant effect from the samplers at the 2 heights ($F_{1,1586} = 0.0198$; $P = 0.888$). However, there was a significant sampler height-spore type interaction ($F_{12,1586} = 12.26$; $P < .001$). Thus, we applied the *t* test to compare the concentrations of individual spore types and found that the mean concentrations of *Alternaria*, ascospores, basidiospores, other spores, *Penicillium/Aspergillus*, and smut spores were significantly different at the 2 levels (Table 1).

Figure 1 shows the concentration of total airborne fungal spores from the 2 Burkard samplers at the 2 heights through-

Table 1. Comparison of the Mean Concentration of Different Airborne Fungal Spores at Rooftop and Ground Heights

Spore type	Mean concentration, spores/m^3		*t* value[a]	*P*
	Rooftop Burkard	Ground Burkard		
Alternaria	244[b]	188	−4.791	<.001
Ascospore	1,587[b]	1,100	−8.653	<.001
Basidiospores	1,144	1,948[b]	9.457	<.001
Cladosporium	4,671	4540	0.345	.36
Curvularia	44	45	0.098	.46
Drechslera	30	22	−2.545	.006
Epicoccum	44	46	1.389	.08
Myxomycetes	34	35	0.334	.37
Nigrospora	29	26	0.092	.46
Other spores	314[b]	196	−6.474	<.001
Penicillium/Aspergillus	470	595[b]	4.140	<.001
Smut spores	301	434[b]	5.799	<.001
Pithomyces	22	20	−1.596	.06
Total spores	8,933	9,197	0.953	.17

[a] Log-transformed values used for statistical analysis.
[b] Significance level $P < .00357$ based on Bonferroni correction.

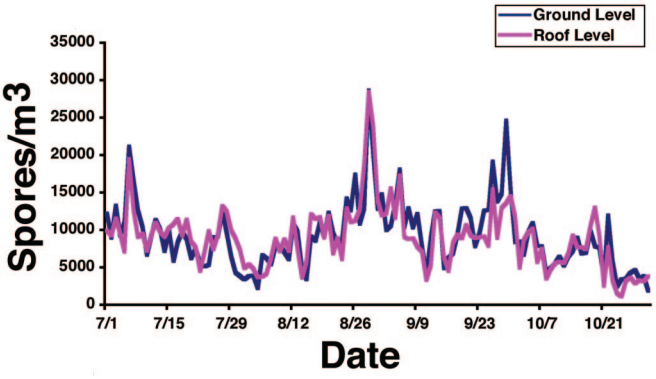

Figure 1. Total airborne fungal spores at 2 different heights.

out the 4 months. The total airborne fungal spore concentration was higher at the ground level on 68 days of the total sampling period of 123 days. The highest difference in the total airborne fungal spore concentrations from the 2 samplers was on September 29, with the concentration on the ground level greater by 11,064. The mean concentration of total airborne fungal spores from the ground Burkard was 9,197 spores/m^3 and that from the rooftop sampler was 8,933 spores/m^3. No significant difference was found between the mean concentration of total airborne fungal spore from the 2 samplers (t =0.953 and P = 0.17) (Table 1).

The concentrations of *Penicillium/Aspergillus* spores, basidiospores, and smut spores were significantly higher on the ground level (Fig 2, Table 1). The mean *Penicillium/Aspergillus* concentration on the rooftop level was 470 spores/m^3, whereas that on the ground level was 595 spores/m^3 (Fig 2a). Basidiospores and smut spores were also significantly higher on the ground level (Table 1). The mean concentrations of basidiospores were 1,948 spores/m^3 and 1,144 spores/m^3 on the ground and rooftop levels, respectively (Fig 2b), and that of smut spores was 434 spores/m^3 on the ground level and 301 spores/m^3 on the rooftop level (Fig 2c).

The rooftop level Burkard captured significantly higher concentrations of *Alternaria,* ascospores, and other spores than those captured at the ground level (Fig 3, Table 1). Figure 3a shows the concentration of *Alternaria* spores from ground and rooftop levels. The mean concentration of *Alternaria* was 244 spores/m^3 at roof level and 188 spores/m^3 on ground. The concentrations of ascospores and other spores at the 2 levels are shown in Figure 3. The roof Burkard collected mean concentrations of 1,587 spores/m^3 ascospores and 314 spores/m^3 of other spores, compared with 1,110 spores/m^3 and 196 spores/m^3, respectively, from the ground Burkard.

No significant differences were found for *Cladosporium*, *Drechslera*, *Nigrospora*, and *Pithomyces* concentrations at the 2 heights (Table 1). These taxa account for 53% of the total concentration on the rooftop level. The mean concentration of *Drechslera* was significantly higher on the rooftop level (P = .006) before applying the Bonferroni correction,

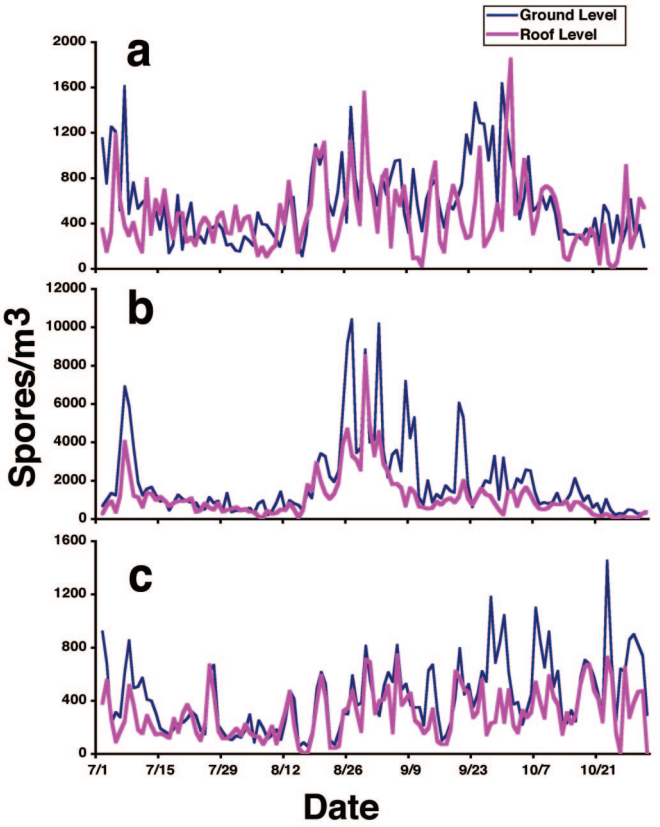

Figure 2. Airborne spore concentrations were significantly higher at ground level. a, *Penicillium/Aspergillus* spores; b, basidiospores; and c, smut spores.

but this spore type represented 0.4% of the cumulative total at the rooftop level.

Positive correlations were found between the concentrations of all types of fungal spores registered by Burkard sampler on the rooftop and those collected by the ground sampler. All the correlations were highly significant except for other spores and myxomycetes (Table 2).

DISCUSSION

The results of this study indicated that the ground level had higher concentrations of some types of airborne fungal spores than the rooftop level. Significantly higher concentrations were found of basidiospores, *Penicillium/Aspergillus*, and smut spores. By contrast, the rooftop Burkard collected significantly higher concentrations of *Alternaria*, ascospores, and other spores (Table 1).

Previous studies have reported basidiospores, *Penicillium/Aspergillus*, and smut spores as important aeroallergens in the atmosphere of many areas, including the Tulsa atmosphere.[3,5,22,34–41] Our results showed that significantly higher concentrations of these important aeroallergens were found on ground level (1.5 m) than on the rooftop level (12 m).

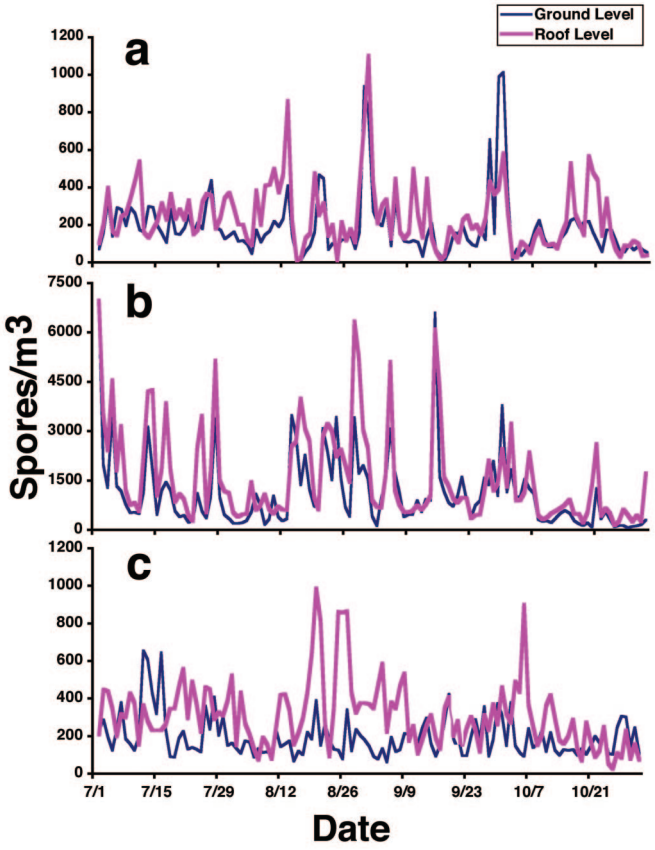

Figure 3. Airborne spore concentrations were significantly higher at rooftop level. a, *Alternaria*; b, ascospores; and c, other spores.

Atluri et al[12] indicated that the concentration of different spore types decreased logarithmically with increasing of height from the ground (0.15 to 4.72 m) above a rice crop in India. The concentration of various spore types decreased in different order at different heights. The results of our study disagreed with the results from this study for the concentration of some spore types, such as *Alternaria,* ascospores, *Cladosporium*, *Drechslera*, *Nigrospora*, *Pithomyces*, and other spores. Also, we have no data below 1.5 m.

Rantio-Lehtimaki et al[10] revealed that concentrations of *Alternaria*, *Cladosporium*, *Epicoccum*, *Drechslera*, and Ustilaginales spores were significantly higher at ground than rooftop level. Uredinales spores were significantly higher at roof level. Our results were only comparable with the results of this study for *Epicoccum* and smut spore concentrations. Chakraborty et al[17] concluded that *Alternaria*, *Curvularia*, and *Drechslera* spore concentrations were highest at 1 m and decreased as the sampling height increased, whereas ascospores, *Aspergillus*-type spores, basidiospores, and *Cladosporium* spore concentrations increased with increasing heights in all seasons. They generally noticed that the smaller spores were commonly found at the high sampling level and large spores were more common at ground level. Our results were dissimilar with their results for some spore types and analogous for other spore types.

Table 2. Correlation Results for the Concentration of Airborne Fungal Spores at Rooftop and Ground Heights

Spores type	Pearson correlation (r^a)
Alternaria	0.7374[b]
Ascospore	0.8091[b]
Basidiospores	0.7706[b]
Cladosporium	0.8830[b]
Curvularia	0.4908[b]
Drechslera	0.4932[b]
Epicoccum	0.4818[b]
Myxomycetes	0.1597
Nigrospora	0.6055[b]
Other spores	0.1434
Penicillium/Aspergillus	0.3356[b]
Pithomyces	0.3605[b]
Smut spores	0.5633[b]
Total spores	0.8140[b]

[a] Log-transformed values used for statistical analysis.
[b] Significance level $P < .00357$ based on Bonferroni correction.

All these studies[10,12,17] suggested that the increase in some fungal spore types and pollen concentrations at the ground level may be a result of the effect of 1 or a combination of several factors. These factors include the proximity to bioaerosol sources (soil and vegetation) at ground, aerodynamic characteristics, size and shape of the sampled bioaerosols, the effect of meteorologic conditions on release, dispersal, and deposition of fungal spores and pollen, and the effect of vertical temperature gradient of the air. These reported factors can explain the significantly lower concentrations of some fungal spores at the rooftop level than the ground level in our study, such as basidiospores, *Penicillium/Aspergillus*, and smut spores.

Some fungal species live on decaying leaves and dead vegetation on the ground. Spore concentrations of these saprophyte species are expected to be higher near ground than rooftop level. This may be a reason for the high concentration of *Penicillium/Aspergillus* spores, which are considered saprophytes and degrade the decayed leaves, and smut spores, which are plant pathogens. Basidiospores concentration was also significantly higher on the ground level possibly because the distance from their source is closer at the ground than the rooftop level.[10]

The effect of meteorologic conditions on release, dispersal, deposition, and concentration of airborne fungal spores was previously reported.[11,36,42–45] Rantio-Lehtimaki et al[10] explained that the long distance transport of bioaerosols may be more obvious at the roof level than the ground level and the wind can cause secondary entrainment of settled pollen and spores, which can be more easily captured at the ground level than rooftop level, but they found that wind speed had no effect on the difference in pollen and spore concentration in their study.

Bergamini et al[9] indicated that *Alternaria* spore concentration was significantly higher at 50 cm above ground in an open field than at 15 m in winter time and was not significantly different at the same height at ground level of an enclosed courtyard in both summer and winter. Our results differed from their study for *Alternaria* concentrations. They concluded that sampling at the rooftop level may underestimate the concentration of some important aeroallergens that may be present in higher concentration at breathing level, such as *Alternaria,* which is considered 1 of the most important aeroallergens worldwide.

The results of our study revealed positive correlations between the concentration of all spore types counted at the ground and that at rooftop levels. The correlations were highly significant except for myxomycetes ($r = 0.1597$, $P = 0.078$) and other spores ($r = 0.1434$, $P = 0.114$) (Table 2). This indicated that airborne spores of most fungal species fluctuated in a similar pattern at both levels most likely in response to meteorologic conditions.

In a recent study[46] *Penicillium* and *Aspergillus* species and total culturable fungi were isolated and identified for 1 year (2007) from ground and rooftop levels. The results of this study revealed that the yearly average concentration of the total culturable fungi was not significantly different at roof and ground levels, which is in agreement with the results presented herein. Also, no significant differences were found between the concentrations of culturable *Aspergillus* and *Penicillium* species isolated at both levels; however, variation was seen in some species of *Penicillium* and *Aspergillus* identified at the 2 heights. The differences between the results of the current study with the culture study for *Penicillium* and *Aspergillus* concentrations at the 2 heights could be due to the differences in sampling method, time, duration, and meteorologic conditions. In the current study, samples were collected 24 hours a day using a spore trap for 4 months during 2005. For the culture study, samples were collected using an Andersen sampler for a 1-minute sampling duration once a week for 1 year (2007).

The results of our study may be not representative of all ground-level locations because the courtyard is an enclosed environment and may be different from an open field or green areas outside the building as was confirmed by Bergamini et al.[9] Our study was conducted for only 4 months during the summer and early fall seasons (July 1 through October 31, 2005). Our study conditions were not similar to those in the previous studies, such as types of air samplers and sampling time and height. In addition, possible differences may be due to the type of plants in the courtyard and the presence of the small stream, which would increase the humidity; Awad[30] reported that type of vegetation in the sampling area can affect the concentration and type of fungal taxa in the atmosphere. These factors may explain the difference between our results and those of other studies.[9,10,12,17] Further research at different levels above the ground for longer time in open areas is recommended to confirm these results.

Aeroallergens are usually monitored by fixed samplers at a certain height (rooftop level) at a single location in cities. Allergists usually rely on the data obtained from these sites for the treatment of patients with allergic diseases; however, the patients may be exposed to different concentrations and types of aeroallergens where they live. The variation in aeroallergen concentrations and types depends on several factors, including the difference in meteorologic factors, sampler type, orifice type, and type of nearby vegetation. We have shown that sampling height is another variable that affects the concentration of some aeroallergens.

In addition, sampling both the indoor and outdoor air of patient homes may be beneficial in some instances. The sampling height should be the same when comparing indoor and outdoor airborne fungal concentrations. For large multistoried buildings, the outdoor reference sample should be collected at the same height as the building air intake. Currently, there are no generally accepted threshold values of spore concentrations that are clinically significant. Therefore, there is no current evidence that the differences shown herein would be recognized by patients. Nevertheless, we recommend allergists still consider these variations in aeroallergen concentrations in the diagnosis and treatment of their patients.

In conclusion, the ground level had significantly higher concentrations of fungal spores of some important aeroallergens, such as *Penicillium/Aspergillus*, basidiospores, and smut spores, which may be underestimated by sampling on the rooftop level. The physician should recognize that the actual exposure by sensitive patients may be different from what is registered by a single regional rooftop sampler. However, sampling at the rooftop level recorded higher concentration of other important aeroallergens, for example, *Alternaria* species. These data show that sampling height is an important variable that influences bioaerosol levels; however, total spore levels were not significantly different at the 2 heights.

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Requests for reprints should be addressed to:
Abeer Khattab, PhD
Biological Science Department
Oliphant Hall
800 S Tucker Dr
The University of Tulsa
Tulsa, OK 74104
E-mail: abeer-khattab@utulsa.edu