Short-Term Variation of the Types and Levels of Outdoor Airborne Fungal Spores Collected at Ground Level

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Abstract

Rationale: Outdoor ground-level air samples (OGLAS) are typically collected over short time intervals while performing indoor building evaluations to allow comparison of the types and levels of fungal spores indoors versus those in OGLAS. This study evaluates the short-term variation of spores in OGLAS.

Methods: 244 pairs of OGLAS were collected during building assessments in Florida (June 2005 – May 2006). The OGLAS were collected before and after indoor samples collection (within an approximate thirty-minute interval), utilizing a Zefon Bio-Pump™ and Air-O-Cell Cassettes™, and analyzed by optical microscopy by the same person. Non-parametric statistical tests were applied.

Results: For paired samples, the agreement ratio of the spore types detected was 0.61 ± 0.18, and the Spearman Rank correlation coefficient (SRCC) was significant in 69.9% of the pairs. Total spores as well as individual Ascospores, Basidiospores, *Cladosporium*, and *Penicillium/Aspergillus* were continuous. The SRCC was significant ($p<0.0001$) for these five spore categories. However, the Wilcoxon test indicated significant differences for total spores, Ascospores, and Basidiospores. The coefficient of variation ranged from 79.6% for Basidiospores to 201% for *Cladosporium*. Eighteen fungal types discontinuously distributed were also detected, primarily in only one of the paired samples.

Conclusions: There is a relatively large variability over short periods of time in the types and levels of fungal spores collected in OGLAS. This variability should be considered in the interpretation of the analytical results of air samples associated with building evaluations and in the design of air sampling protocols.

Objective

The purpose of this study is to determine the short-term temporal variation of the types and levels of outdoor airborne fungal spores in pairs of samples collected from the same location at ground level over short periods of time throughout a 12-month period in Florida.

Materials and Methods

Collection of Air Samples

488 outdoor air samples were collected from June 2005 through May 2006 during the course of 244 building assessments throughout Florida, utilizing a Zefon Bio-Pump™ and Air-O-Cell Cassettes ™ (Zefon International Inc., Ocala, Florida, USA). Samples were collected from the exterior of the buildings approximately one meter above the ground at a flow rate of 15 liters per minute for 5 minutes. One sample was collected prior to and one sample after the collection of the indoor air samples, within approximately a 30-minute time interval. All samples were collected during daylight hours, approximately from 8 am through 5 pm.

Non-Culturable Analysis of the Air Samples

All samples were analyzed by optical microscopy by the author. The glass slides contained within the cassettes were removed, taped onto regular microscope glass slides, treated with lactic acid, and analyzed as follows:

- The deposit trace was located under a magnification of 100X.
- Large spores (> 7 µm) present on 100% of the deposit trace were identified and counted under a magnification of 400X.
- Small spores (< 7 µm) and/or spores detected in large amounts present on 25% of the deposit trace were identified and counted under a magnification of 1,000X with immersion oil.
- Spores were identified at the genus level or classified into groups following currently accepted taxonomic guidelines.
- The results of total spores and specific spore types were expressed as spores/m^3 of air.
- The amount of background debris was recorded semi-quantitatively utilizing a scoring system ranging from 1+ to 3+.

Statistical Analysis

Statistical analysis was performed utilizing the Analyze-it Software™ (Analyze-it Software Limited, Leeds, United Kingdom) as follows:

- The Kolmogorov-Smirnov test was utilized to ascertain the normality of the variables with continuous distributions.
- Non-parametric descriptive statistics were calculated.
- The Spearman Rank correlation coefficient and the Agreement Ratio were calculated for each of the 244 pairs of samples to evaluate the concordance of the fungal types detected between paired samples.

Several tests (Wilcoxon Signed Rank test, coefficient of variation utilizing the Method Error, and Spearman Rank correlation coefficient) were also utilized to compare the spore types with continuous distributions between both sets of outdoor samples.

Note: A p value <0.05 was considered significant in these analyses

General Spore Diversity

Twenty-two different types of fungal spores, all non-normally distributed, were identified in the air samples. The diversity between sample pairs was as follows:

- Spearman Rank Correlation Coefficient: Significant in 69.9% of paired samples
- Agreement Ratio: 0.61 ± 0.182

Spores with Continuous Distributions

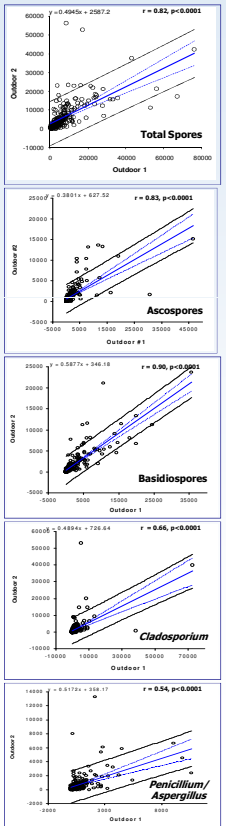
Total spores as well as individual Ascospores, Basidiospores, *Cladosporium*, and *Penicillium/Aspergillus* were continuously distributed in both sets of samples. These spore types were detected in the majority of samples (N = 244 paired samples).

Spore Type	Absent in Both Samples	Present in 1 Sample	Present in Both Samples
Total Spores	*0.0 %*	*0.0 %*	*100.0 %*
Ascospores	*4.9 %*	*13.9 %*	*81.1 %*
Basidiospores	*4.5 %*	*10.2 %*	*85.2 %*
Cladosporium	*1.6 %*	*14.7 %*	*83.6 %*
Penicillium/Aspergillus	*0.8 %*	*9.4 %*	*89.7 %*

A large quantitative variation between both sets of samples was detected. The greatest agreement between paired samples was detected for total spores, Ascospores, and Basidiospores; the least for *Cladosporium* and *Penicillium/Aspergillus*.

Spore Type	Coefficient of Variation (CV)	Wilcoxon Signed Rank Test (p value)
Total Spores	*82.1 %*	*0.006*
Ascospores	*144.6 %*	*0.003*
Basidiospores	*79.6 %*	*< 0.0001*
Cladosporium	*201.0 %*	*0.1*
Penicillium/Aspergillus	*118.4 %*	*0.89*

Regression between Paired Samples



Spores with Discontinuous Distributions

Eighteen types of fungal spores discontinuously distributed were detected in the samples. Many of these fungal types were either absent in both paired samples or detected in one sample only. *Chaetomium* and *Stachybotrys* (moisture-indicator fungi) were the only spore types that were never detected in two paired samples (N = 244 pairs).

Spore Type	Absent in Both Samples	Present in 1 Sample	Present in Both Samples
Curvularia	*16.4 %*	*24.6 %*	*59.1 %*
Smuts/Periconia/Myxomycetes	*13.1 %*	*23.8 %*	*63.1 %*
Alternaria	*46.7 %*	*31.1 %*	*22.1 %*
Bipolaris/Drechlera	*52.9 %*	*31.1 %*	*16.0 %*
Botrytis	*89.7 %*	*7.4 %*	*2.9 %*
Cercospora	*61.1 %*	*27.0 %*	*11.9 %*
Chaetomium	*95.9 %*	*4.1 %*	*0.0 %*
Epicoccum	*92.2 %*	*4.5 %*	*3.3 %*
Nigrospora	*38.1 %*	*34.4 %*	*27.5 %*
Mildew (Peronospora, Oidium)	*74.2 %*	*22.1 %*	*3.7 %*
Pithomyces	*65.2 %*	*25.0 %*	*9.8 %*
Rusts	*73.8 %*	*20.1 %*	*6.2 %*
Spegazzinia	*83.6 %*	*14.3 %*	*2.0 %*
Stachybotrys	*97.9 %*	*2.0 %*	*0.0 %*
Stemphylium	*99.1 %*	*4.1 %*	*0.8 %*
Tetraploa	*91.0 %*	*7.4 %*	*1.6 %*
Torula	*77.4 %*	*18.8 %*	*3.7 %*
Ulocladium	*90.0 %*	*9.4 %*	*0.4 %*

Conclusions

Total spores as well as individual Ascospores, Basidiospores, *Cladosporium* and *Penicillium/Aspergillus* have continuous, skewed distributions. While the quantitative variation of the levels of these fungal types is large, paired samples are significantly correlated. Therefore, one outdoor air sample could be sufficient for comparison purposes with indoor air samples collected during building evaluations.

Eighteen types of fungal spores with discontinuous distributions were detected. The qualitative variation of the majority of these spore types is large, and they were primarily detected in one of the paired samples only. Therefore, various outdoor samples should be collected to detect the presence of these spore types. Ideally, statistical models should be utilized for different spore types to predict probabilities of detection.

Chaetomium and *Stachybotrys* (moisture indicator fungi) were only sporadically detected in outdoor samples. Therefore, a large number of outdoor air samples should be collected during the course of each building assessment. Because this approximation is impractical, the recommended comparison of spore types and levels detected indoors vs outdoors should not be performed for these spore types. The mere presence of these fungal types in indoor air samples should be considered relevant regardless of the presence/absence and level of these fungal types outdoors.

Further studies should be performed to properly address the impact of specific environmental factors responsible for short-term variations of outdoor fungal spore types and levels collected at ground level. This information would facilitate the design of site-specific sampling protocols depending on the environmental conditions present at the time of sampling.