Typical Levels of Airborne Fungal Spores in Houses Without Obvious Moisture Problems During a Rainy Season in Florida, USA

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Abstract

Objective: The aim of this study was to determine types and levels of airborne fungal spores in air-conditioned homes built after 1980 without obvious moisture problems during the 2004 summer (rainy season) in central Florida, USA.

Methods: Eighteen single-family homes were selected based on protocol questionnaire and cursory inspection, which revealed no obvious moisture or visible fungal growth. Non-cultured spores were collected with Air-O-Cell cassettes. Three indoor air samples and 2 outdoor air samples were collected from each home. One indoor and 2 outdoor samples were not interpretable. Fifty-three indoor and 34 outdoor air samples were analyzed by optical microscopy.

Results: Several spore types were detected in the indoor samples, at levels generally lower than those detected in the outdoor samples. Spores from the *Penicillium/Aspergillus* group were the most prevalent types indoors, exceeding the absolute levels and relative percentages of these spores outdoors. Ascospores and basidiospores were the most prevalent spore types outdoors. The percentages of other spore types (*Cladosporium* and *Curvularia*) were similar in the indoor and outdoor samples. Moisture-indicator fungi (*Chaetomium, Stachybotrys,* and *Ulocladium* species) were nearly absent in both indoor and outdoor samples.

Conclusion: Airborne fungal spores are present in average central Florida homes without obvious moisture problems during the summer, at levels that are lower than those found outdoors. Spores from the *Penicillium/Aspergillus* group are prevalent in these homes, and moisture-indicator fungi (*Chaetomium, Stachybotrys*, and *Ulocladium* species) are nearly absent. Despite climatic differences, airborne fungal spore types and levels in central Florida houses are similar to those found in other geographical locations.

Key words: Mold. Fungal spores. Background levels. Moisture problems.

Resumen

Objetivo: Este es un estudio para determinar los tipos y las concentraciones de esporas fúngicas atmosféricas en las casas que disponen de aire acondicionado, construidas después de 1980 sin problemas de humedad evidentes, durante el verano de 2004 (estación de lluvias), en Florida central, EE.UU.

Métodos: Se seleccionaron dieciocho casas unifamiliares en función de un cuestionario de protocolo y una inspección superficial que revelaron que no existían ni una humedad evidente, ni infestación por hongos visible. Las esporas totales se recogieron con cassettes Air-O-Cell. También se recogieron de cada casa tres muestras de aire del interior de la casa y 2 del exterior, de los que no se pudieron interpretar una muestra interior y 2 exteriores. Se analizaron mediante microscopio óptico cincuenta y tres muestras de aire interior y 34 muestras exteriores.

Resultados: En las muestras de aire interior se detectaron varios tipos de esporas en unas concentraciones generalmente menores que las detectadas en las muestras de aire exterior. Las esporas del grupo *Penicillium/Aspergillus* fueron los tipos más prevalentes en las muestras de interior y excedían las concentraciones absolutas y los porcentajes relativos que se pueden encontrar en las muestras exteriores. Los tipos de esporas más prevalentes en las muestras de aire exterior fueron las ascosporas y las basidiosporas. Los porcentajes de otros tipos de esporas (*Cladosporium y Curvularia*) fueron similares en las muestras interiores y en las exteriores. Los hongos indicadores de humedad (especie *Chaetomium, Stachybotrys y Ulocladium*) fueron prácticamente inexistentes en ambos tipos de muestras.

Conclusión: Las esporas fúngicas atmosféricas están presentes en las casas típicas de Florida sin problemas de humedad evidentes durante el verano, a concentraciones que son más bajas que las que se pueden encontrar al aire libre. Las esporas del grupo Penicillium/Aspergillus son prevalentes en estas casas y los hongos indicadores de humedad (especie *Chaetomium, Stachybotrys y Ulocladium*) son prácticamente inexistentes. A pesar de las diferencias climáticas, los tipos y las concentraciones de esporas fúngicas atmosféricas en las casas de Florida central son similares a las que se pueden encontrar en otras localizaciones geográficas.

Palabras clave: Moho. Esporas fúngicas. Concentraciones de fondo. Problemas de humedad.

Introduction

The high prevalence of allergies in developed countries contributes to the increased public concern about indoor air quality. This has resulted in an increase in demand for environmental assessments, which at the present time are mainly focused on assessment of buildings for evidence of indoor fungal growth (mold).

Samples are sometimes collected from air or surfaces during building assessments, and fungal spores are identified using non-culturable or culturable analysis as a surrogate measure of exposure to fungal allergens. Individuals often bring the results of this testing to their physician for interpretation. However, scientifically valid methods for data interpretation are currently unavailable. Dose-response relationships between exposure to fungi and symptoms are lacking. While a number of arbitrary numeric standards for "acceptable" levels of indoor fungi have been proposed [1], none of them are currently accepted by the scientific community.

Although several studies conducted in different geographical locations around the world report "background" levels of fungal spores in buildings, these studies are limited in design and the majority of them do not specifically select buildings for the absence of fungal growth or moisture problems [2-18]. Welldesigned studies that propose baseline levels for fungal spores in buildings without obvious moisture problems are scarce [19]. However, recommendations for data interpretation have been suggested [20,21]. The evaluation of air sampling results is currently based on the comparison of the types and levels of fungal spores detected indoors versus those detected in matched outdoor samples. Indoor types of airborne spores should be similar to those detected outdoors and the levels should be lower. A critical consideration in the interpretation of air sampling results is the fact that indoor levels of airborne spores are always mixtures of spores from the following sources: infiltration of outdoor air, disturbance of dust reservoirs containing settled spores within a building, building occupants, and indoor fungal growth (both "minor and typical growth" and that occurring as a result of moisture problems). Which factors prevail depends on the specific conditions at the time of sampling.

Geographical location, climate, and short-term meteorological conditions are responsible for outdoor types and levels of fungal spores. Geographical location and climate are also responsible for particular construction practices, which partially account for the types and levels of indoor airborne fungal spores. Therefore, it is reasonable to assume that background levels of indoor airborne fungal spores may vary according to location. Moisture is a limiting factor for fungal growth. Because of Florida's subtropical location, seasonal climatic variations include abrupt changes in rainfall and ambient humidity levels between 2 seasons, the rainy season (from approximately June through October) and the drier season (during the remaining months of the year). It is unknown whether houses without obvious moisture problems in subtropical areas have higher levels of airborne spores than those located in other climatic locations as a result of infiltration of outdoor humid air during summer months. The objective of this pilot study was to investigate types and levels of airborne fungal spores by nonculturable sampling in air-conditioned houses without obvious moisture problems during a rainy season in Florida, USA.

Materials and Methods

Selection of Houses

Ethical approval was obtained from the Institutional Review Board of University of South Florida Health Science Center in Tampa, Florida, USA. Volunteers were informed about the purpose of the study and the entry criteria, and a consent form was signed. In order to be eligible for the study, houses were required to meet the following entry criteria: (1) single-family detached house; (2) built after 1980; (3) house between 1500 and 2500 square feet; (4) homeowners are annual residents; (5) at least 1 carpeted area frequently used; (6) no air purifiers or dehumidifiers used; (7) central air conditioning used throughout most of the year; (8) no visible fungal growth identified on any surface within the house since it was purchased; (9) no current or past water leaks, flooding, or moisture problems; and (10) no musty odors noticed within the house. Restricting the group of houses to those that met these 10 criteria helped to reduce variability due to housing type and to focus on the average characteristics of houses present in central Florida.

Eighteen homes met the entry criteria and each was enrolled in the study, which was performed during the summer of 2004 in Tampa, Florida, USA. A basic questionnaire including questions regarding the home and demographic characteristics was administered to each homeowner.

Inspection of Houses

An assessment of each house enrolled in the study was performed to evaluate obvious moisture problems. During the course of the assessments, a visual inspection of the houses was performed to identify the potential presence of fungal growth and sources of water intrusion or water loss. Additionally, temperature and relative humidity were measured during the course of the site inspections using an electronic humidity and temperature meter to investigate the potential existence of condensation problems as a result of elevated relative humidity.

Sampling of Houses

Air samples were collected for non-culturable analysis during the site inspection utilizing a battery operated pump and collecting cassettes (Zefon Bio-Pump and Air-O-Cell cassettes, Zefon, Ocala, Florida, USA) collected for 15 minutes at a flow rate of 15 L/min, approximately 1 m above floor level. One sample each was collected from the master bathroom, kitchen, and a frequently used carpeted room. Two outdoor air samples were also collected from the driveway for comparison purposes, 1 before and 1 after the collection of indoor samples.

Sample Analysis

All samples were analyzed by one of the authors (R.C.). The analysis was performed by bright field optical microscopy, using a microscope equipped with $10 \times, 40 \times,$ and 100× objectives (Motic B300, Motic Instruments Inc, Richmond, British Columbia, Canada). The glass slides contained within the cassettes were removed, taped onto regular glass microscope slides, treated with lactic acid, and analyzed as follows: the particle deposit trace was located at a magnification of $100 \times$; large spores (> 7 µm) present on 100% of the deposit trace were identified and counted at a magnification of 400 \times ; small spores (< 7 μ m) and spores detected in large amounts present on 25% of the deposit trace were identified and counted at a magnification of 1000×. Spores were identified at the genus level or classified into groups following general taxonomic guidelines currently accepted by the scientific community. The results of total spores and specific spore types were expressed as spores/ m³ of air. The air samples were also examined specifically for the presence of spores that would be indicative of indoor fungal growth, which consisted of Chaetomium, Stachybotrys, and Ulocladium species (moisture indicator fungi).

Statistical Analysis

Nonparametric statistical analysis was performed with Analyze-it software (Analyze-it Software Limited, Leeds, UK). Nonparametric descriptive statistical parameters were calculated for the spore types with continuous distributions. The Kolmogorov-Smirnov test was used to ascertain the normality of the variables with continuous distributions. Rankings of abundance of spore types detected in indoor and the corresponding outdoor air samples were compared using the Spearman rank correlation coefficient. Rankings of abundance of particular spore types detected in the sets of 3 indoor air samples were compared using the Friedman test. A *P* value less than .05 was considered significant.

Results

House Characteristics

None of the 18 houses sampled had fungal growth or obvious moisture problems, as determined by homeowner survey and inspection. The average house characteristics are described in Table 1. All but 1 of the indoor relative humidity levels were below 60%, the upper level of relative humidity recommended to prevent fungal growth.

Air Sampling

One indoor and 2 outdoor samples were not interpretable. Therefore, the results of 53 indoor and 34 outdoor air samples were analyzed. Fungal spores were detected in all samples (Table 2). A large diversity of spore types with a broad dispersion of values was found in all samples. The most abundant spore types present both indoors and outdoors were generally *Penicillium/Aspergillus* group, ascospores, basidiospores, *Cladosporium* species, spores classified in the Smuts/*Periconia/* Myxomycetes group, and *Curvularia* species (Table 3). These spore types had continuous, skewed distributions as determined by the Kolmogorov-Smirnov test.

Penicillium/Aspergillus was the most prevalent fungal type detected in the indoor air samples (Tables 2 and 3). They were detected in 52 of 53 (98.1%) samples and accounted for the majority of the spores detected indoors (median = 52% of total spores). The indoor/outdoor ratio of these spore types varied considerably among samples, with a median of 0.78.

Table	1	Home	Characteristics
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	Square Feet	Age, y	Height in Stories	Number of Pets	Carpeted Area, %	Number of Occupants	Temperature, °C (°F) ^a	Relative Humidity, % ^b
Median	2001	3.5	1	0	75.0	3	26.5 (79.7)	42.1
Range	1500-2948	0.3-16	1-3	0-2	10-90	2-5	24.4-28.6 (76.0-83.4)	32.4-57.6

^a Outside temperature, median = 82.3°F (79°F-95.8°F)

^bOutside relative humidity, median = 65.3% (51.8%-83.2%)

Ascospores and basidiospores were the most prevalent fungal types detected in the outdoor air samples (Tables 2 and 3). These spore types were detected in all samples and accounted for the majority of the spores detected outdoors (median ascospores = 32.3% of total spores, median basidiospores = 24.7% of total spores). In contrast, spores from the *Penicillium/Aspergillus* group accounted for a minor proportion of the total spores detected in the outdoor air samples (median = 6.6% of total spores).

Eighteen additional fungal types were detected in the outdoor air samples. Ten of these fungal types were also detected in the indoor air samples (Table 4). Moisture indicator fungi were essentially absent, particularly from the Table 2. Prevalence of Total Spores and the Most Abundant Spore Types in Indoor (n=53) and Outdoor (n=34) Samples

Spore Type	Indoor, No. (%)	Outdoor, No. (%)
Penicillium/Aspergillus grou	p 52 (98.1)	30 (88.2)
Ascospores	51 (96.2)	34 (100)
Basidiospores	34 (64.1)	34 (100)
Cladosporium	33 (62.3)	33 (94.1)
Smuts/Periconia/Myxomycete	s 38 (71.7)	33 (97.0)
Curvularia	32 (60.4)	28 (82.3)

	Table 3. Most Abundant S	oore Types in Indoor ((n=53) and Outdoor	(n=34) Samples ^a
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Spore Type	Indoor Spores/m ³	Outdoor Spores/m ³	% of Total Indoor Spores	% of Total Outdoor Spores	% of Indoor vs Outdoor Spores
Total	335 (8-999)	2355 (468-333 538)	_	_	11.0 (0.06-61.5)
Penicillium/Aspergillus	194 (0-656)	176 (0-3250)	52 (13.6-91.7)	6.6 (0.08-28.2)	77.8 (0.6-754.8)
Ascospores	53 (0-317)	688 (123-176 000)	16.7 (0.02-50.0)	32.3 (4.9-73.9)	3.3 (0-55.5)
Basidiospores	9 (0-279)	726 (70-156 992)	3.2 (0.05-41.3)	24.7 (0.5-61.9)	1.0 (0-26.7)
Cladosporium	18 (0-176)	141 (0-3447)	6.1 (2.0-55.7)	5.1 (0.03-36.4)	4.9 (0-166)
Smuts/Periconia/Myxomycetes	9 (0-106)	123 (0-7289)	3.7 (0.7-50.0)	7.6 (0.05-48.0)	4.5 (0-158)
Curvularia	4 (0-88)	20 (0-1866)	1.5 (0.06-37.7)	1.1 (0,02-19.2)	6.3 (0-367)

^a Data are shown as medians (range)

indoor samples. *Stachybotrys* and *Ulocladium* species were detected in only 1 each of the 53 (1.9%) indoor samples and in 1 (2.9%) and 6 of the 34 (17.6%) of the outdoor samples, respectively.

The Friedman test was applied for 17 houses (all houses except the house in which only 2 indoor air samples were collected) and was not significant (P > .05) in 13 of 17 (68.7%) of the sets of indoor samples, indicating a similar diversity of fungal types in the 3 samples comprising each set. The Spearman rank correlation coefficient was statistically significant (P < .05) in the 3 indoor samples collected in 8 of the 18 (44%) houses, 2 of the 3 indoor samples collected in 4 of the 18 (22%) houses, and 1 of the 3 indoor samples collected in 4 of the 18 (22%) houses, indicating a general agreement in the spore types and levels detected indoors versus those detected in the corresponding outdoor samples.

Discussion

While various studies that identify types and levels of airborne fungal spores in buildings have been performed, the majority of them focus on problem buildings with fungal

Table 4. Prevalence of Other Spore	Types in Indoor	(n=53) and	d Outdoor
(n=34) Samples ^a			

Spore Type	Indoor Samples	Outdoor Samples
Nigrospora	16 (30.2)	16 (47.0)
Bipolaris/Drechlera	6 (11.3)	12 (35.3)
Spegazzinia	5 (9.4)	10 (29.4)
Alternaria	6 (11.3)	9 (26.5)
Stemphylium	_	6 (17.6)
Torula	1 (1.9)	6 (17.6)
Ulocladium	1 (1.9)	6 (17.6)
Pithomyces	3 (5.7)	6 (17.6)
Peronospora, Oidium	_	4 (11.8)
Rusts	_	3 (8.8)
Botrytis	_	2 (5.9)
Tetraploa	_	2 (5.9)
Epicoccum	5 (9.4)	2 (5.9)
Cercospora	_	2 (5.9)
Stachybotrys	1 (1.9)	1 (2.9)
Trichoderma	_	1 (2.9)
Fusarium	_	1 (2.9)
Myrothecium	1(1.9)	1 (2.9)

^a Data are shown as number (%).

growth, moisture problems, or health complaints from building occupants [9,12,16,18]. Other studies have attempted to include reference buildings without moisture problems to determine background levels of fungal spores indoors [7-15]. However, buildings were not specifically selected for the absence of visible fungal growth and obvious moisture problems, and some of these studies included both commercial and residential buildings [8-17]. In addition, the majority of these studies used the results of commercially analyzed samples, presumably collected by building inspectors from buildings under investigation following complaints, and probably used different sampling equipment and sampling protocols. While non-culturable analysis is currently the most common type of analysis performed during the course of building assessments and seen by the physician, the majority of these studies exclusively performed culturable analysis. Gots et al [22] compiled a number of reports that included measurements of fungi from sites including reference buildings without occupant complaints. This review utilized total counts for comparison purposes and failed to consider any ecological grouping of fungi.

Horner et al [19] performed a study in Atlanta, Georgia, USA, an area with similar climatic characteristics to those present in Florida, and specifically preselected the houses included in the study for the absence of fungal growth and moisture problems, as was done in this study. While samples were collected for culturable analysis, consistent sampling equipment and protocols were used and indoor samples were collected from locations similar to those sampled in this study. To our knowledge, ours is the first study in which air samples collected from houses previously preselected for the absence of fungal growth or moisture problems were analyzed for non-culturable spores. None of the houses included in this study had visible signs of fungal growth or moisture problems. In addition, all but 1 of the indoor relative humidity levels were below 60%. The US Environmental Protection Agency [23] and the American Conference of Governmental Industrial Hygienists [24] have published guidelines in which maintenance of relative humidity at levels below 60% is suggested as a measure to prevent fungal growth. While factors exist that may contribute to fungal growth in areas with relative humidity below 60%, it is generally accepted as the upper end of the range of desired levels.

All samples, collected using the same equipment and sampling protocol throughout the study, were analyzed by the same person, thus reducing part of the variation associated with sample collection and analysis. Despite this consistency in sample collection and analysis, a large variation of fungal concentrations was detected, particularly in outdoor samples. This variation should be considered in the interpretation of non-culturable air sampling reports.

Despite differences in analytical protocols, the results of this study are consistent with those reported by Horner et al [19], who grouped fungi according to categories with different ecological relevance as phyloplane fungi (leaf surface fungi), soil fungi, and water-indicator fungi. Phyloplane fungi included *Cladosporium, Curvularia*, and *Alternaria* species; soil fungi included *Penicillium, Aspergillus*, and *Paecilomyces* species, which produce spores classified in the group of *Penicillium/Aspergillus* by non-culturable analysis; and water-indicator fungi included *Chaetomium, Stachybotrys,* and *Ulocladium* species.

In this study, the most prevalent spore types detected in both the indoor and outdoor air samples were generally from the *Penicillium/Aspergillus* group, ascospores, basidiospores, and *Cladosporium* species. These findings are qualitatively similar to those observed in other geographical locations [11,14,17-19,25,26], confirming the ubiquitous nature of these fungi.

This study also indicates a similarity of fungal types and concentrations within portions of buildings, as well as a similar diversity of fungal types indoors versus those detected outdoors, supporting general interpretative guidelines. However, spores classified in the group of *Penicillium/Aspergillus* species, which were the most prominent spore types in the indoor air samples, generally exceeded the relative percentage of these spore types detected outdoors. Ascospores and basidiospores, which were the most abundant spore types in the outdoor air samples, were detected at much lower concentrations indoors. These spores do not commonly grow in buildings, and their detection in indoor samples typically suggests infiltration of outdoor air (and the opposite when these spore types are low or absent).

Penicillium, Aspergillus, and other related genera of fungi commonly amplify indoors when there are moisture problem. Therefore, particular attention is typically given to the levels of these fungal types detected indoors versus those detected outdoors, and, as previously suggested, the ratio of soil/phyloplane fungi should be considered a measure of fungal growth within buildings [19]. However, it should be noted that these fungi also commonly grow in house dust in buildings without obvious moisture problems and can be detected indoors at levels greater than those detected outdoors [27,28], as illustrated by the results of this study. This suggests that the contribution of infiltration of outdoor air to the levels of spores detected indoors was minimal. Therefore, the indoor/ outdoor ratio for the Penicillium/Aspergillus group should be considered with caution in the interpretation of non-culturable airborne fungal reports, particularly when outdoor levels of these fungal types are low, as defined by regional outdoor aerobiology surveys [29].

Low levels of other genera or groups of fungi were also identified. While this study was performed in a subtropical location and during a rainy season, moisture-indicator fungi, including *Chaetomium*, *Stachybotrys*, and *Ulocladium* species were nearly absent in the samples, particularly in those collected indoors. These findings are similar to those obtained in other areas of the USA, where studies failed to detect the presence of these spore types in urban buildings or in the outdoor environment [19,30,31].

The presence of airborne spores produced by these moisture indicator fungi typically suggests the presence (or prior presence) of damp materials in the vicinity of the air sample location that have likely been exposed to moisture for an extended period of time. It should be noted that these fungi do not normally release high levels of spores and that these spore types do not remain airborne for extended periods of time. It should also be noted that these spores are not normally present outdoors in high numbers, as indicated by the results of this study. Therefore, relatively low levels of these spore types (compared to other types of spores that are released in large amounts, such as those from *Penicillium/Aspergillus* species) may indicate an amplification or elevation of fungal spores. Consequently, the presence of these spore types in indoor air samples should be considered relevant in the interpretation of laboratory reports of airborne fungal spores regardless of the presence or absence of these fungal types in outdoor air samples. This consideration is based on fungal ecology, familiarity with different types of spores, and personal experience rather than on potential health effects caused by moisture indicator fungi, which are beyond the scope of this study.

In conclusion, this pilot study reports general background levels of fungal spores in houses without obvious moisture problems during a rainy season in Florida and, by extension, in other subtropical locations. Similar studies should be conducted in other geographical locations and during different seasons of the year. This study also confirms the validity of general interpretative guidelines, but suggests that indoor/ outdoor ratios of airborne fungal spores should be evaluated in conjunction with other factors, including ecological relevance of fungi, mechanisms of spore formation and release, and aerodynamic characteristics of spores.

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