ORIGINAL ARTICLE

Guidelines on Ambient Intramural Airborne Fungal Spores

A Fairs[1], AJ Wardlaw[1], JR Thompson,[2] CH Pashley[1]

[1]Aerobiology Unit, Institute for Lung Health, Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom
[2]Centre for Biostatistics and Genetic Epidemiology, Department of Health Sciences, University of Leicester, Leicester, United Kingdom

Abstract

Objectives: To generate baseline data for indoor airborne fungal spores in noncomplaint residential properties (with no moisture/mold-related problems) and to identify home characteristics indicative of elevated fungal levels.
Methods: Air samples were collected onto petroleum jelly–coated slides from living rooms of 100 residential properties in Leicestershire, United Kingdom, using a Burkard continuous recording air sampler. The slides were examined by microscopy to determine fungal spore concentrations (spores/m^3 air/day).
Results: Total indoor fungal spore concentrations were approximately 16% of outdoor concentrations. Abundant indoor fungal genera include *Cladosporium, Sporobolomyces, Tilletiopsis*, and *Didymella*, all of which followed seasonal patterns of release and detection. No clear association was shown between outdoor-predominant fungi and home characteristics. In contrast, *Aspergillus/Penicillium*-type (*Asp/Pen*-type) spores were common indoors and exceeded outdoor levels, with the highest concentrations detected in properties over 90 years old (P=.006) and terraced properties (P=.003).
Conclusion: Asp/Pen-type spores are found in noncomplaint UK residential properties and mostly in old terraced houses. This study provides guidelines on acceptable levels of *Asp/Pen*-type spores and other abundant indoor fungal taxa that can be comparatively used in clinical evaluations of fungal exposure–related disease.

Key words: Mold/mould. Fungal spores. Environmental exposure. Reference values. Aerobiology

Resumen

Objetivos: Generar valores de referencia de esporas fúngicas en el aire interior en propiedades residenciales en buen estado (sin problemas relacionados con humedades o moho) e identificar las características del hogar indicativas de niveles fúngicos elevados.
Métodos: Se obtuvieron muestras de aire en portaobjetos recubiertos con vaselina de los salones de 100 propiedades residenciales en Leicestershire, Reino Unido, utilizando un muestreador de aire Burkard de registro continuo. Los portaobjetos se examinaron mediante microscopía para determinar las concentraciones de esporas fúngicas (esporas/m^3 de aire/día).
Resultados: Las concentraciones totales de esporas fúngicas en el interior fueron aproximadamente el 16% de las concentraciones del exterior. Los géneros de hongos de interior más abundantes fueron *Cladosporium, Sporobolomyces, Tilletiopsis* y *Didymella*, y todos ellos siguieron patrones estacionales de liberación y detección. No se observó una relación clara entre los hongos predominantes en el exterior y las características del hogar. En cambio, las esporas de tipo *Aspergillus/Penicillium* (tipo *Asp/Pen*) fueron frecuentes en el interior y superaron los niveles del exterior; las concentraciones más elevadas se detectaron en propiedades de más de 90 años de antigüedad (p=0,006) y propiedades con terraza (p=0,003).
Conclusión: Las esporas de tipo *Asp/Pen* se encuentran en propiedades residenciales en buen estado del Reino Unido, principalmente en casas antiguas con terraza. Este estudio proporciona guías sobre los niveles aceptables de esporas de tipo *Asp/Pen* y otros taxones fúngicos de interior abundantes que pueden utilizarse comparativamente en evaluaciones clínicas de enfermedades relacionadas con la exposición fúngica.

Palabras clave: Moho. Esporas fúngicas. Exposición ambiental. Valores de referencia. Aerobiología.

Introduction

Adverse health effects associated with fungal exposure are widely documented and extremely diverse, ranging from headaches to allergy and invasive infections [1]. Fungi are ubiquitous, causing respiratory allergy in 20% to 30% of atopic individuals [2]. The majority of spores are 2 to 10 μm in size [3] and can easily penetrate the lower airway, with major allergic manifestations including asthma, rhinitis, and allergic bronchopulmonary mycoses [2]. More specifically, sensitization to fungal genera including *Alternaria*, *Aspergillus*, and *Cladosporium* has been associated with asthma severity [4].

Building construction has been adapted to facilitate more energy-efficient housing and moved towards the use of cellulose-based materials, which are easily digested by fungi [3], coinciding with increased time spent inside buildings in industrialized countries and an increase in the prevalence and morbidity of asthma and allergy [5,6].

Housing characteristics reported as predictors of fungal contamination include relative humidity (RH) >80%, temperature, season, presence of cats, old carpets, highly insulated windows, central heating, and wooden board flooring [3,7]. Visible fungal (mold) growth has been linked with elevated *Aspergillus* and *Penicillium* concentrations [8], which are common indoors and contain species with known adverse effects on health. However, nonvisible mold growth concealed within wall cavities or building materials can also negatively affect indoor air quality [9].

Assessing the dose-response relationship between fungal exposure and respiratory symptoms requires objective measures of fungal levels within properties [8,10]. It is still not possible to provide guidelines on acceptable levels of fungal exposure due to inconsistencies in study protocols, data reported, and validations of health outcomes [11,12]. Many studies of indoor fungal contamination have used culture as a means of quantification and identification but this technique has certain limitations such as short sample duration, biased identification due to preferences in growth media, and detection only of viable spores. An alternative method of fungal quantification is the collection of air samples on microscope slides and subsequent analysis by microscopy using standardized techniques, which do not have the bias of viability [13-15]. Indoor fungal concentrations, taxa, and exposure thresholds are essential for evaluating the health impacts of exposure to fungi [16].

Prior to analyses of complaint or "atypical" properties, where a causative effect on health is being suggested, robust regional baseline data of "typical" indoor fungal exposure must be collected [17]. The objective of this study was to determine airborne fungal spore concentrations in noncomplaint residential properties (with no moisture/mold-related problems) and to identify home characteristics predictive of elevated fungal levels. This study provides a comprehensive resource that can be used for comparative purposes in future studies monitoring indoor air quality, assessing fungal exposure, determining fungal remediation successes, and assisting in medical evaluations of a causative effect of fungal exposure on health.

Materials and Methods

Selection of Residential Properties

Air samples were taken from the living rooms of 124 properties within Leicestershire from August 2006 to January 2008. Volunteers were recruited from the University of Leicester, University Hospitals of Leicester and associates. Properties sampled were defined as noncomplaint in that there were no suspected mold or damp problems within the property and no known health effects due to residential air quality. Property characteristics were collected using a detailed questionnaire to investigate potential influences on airborne fungal spore concentrations.

Air Sample Collection and Analysis

Air samples were collected at a constant flow rate of 10 L/min onto petroleum jelly-coated slides moving at a constant speed of 2 mm/h to produce a 24-hour trace, using a continuous recording air sampler (Burkard Manufacturing Co, Rickmansworth, UK) placed approximately 0.5 to1.0 m above the floor. Slides were stained with polyvinyl lactophenol cotton blue and analyzed by microscopy at a magnification of 630×. A single longitudinal transverse of 1 field width was counted for each sample, as described previously [18]. Distinct spore morphology distinguished 17 fungi to the level of genus. The other fungi were categorized into closely related groups, such as *Aspergillus/Penicillium* (*Asp/Pen*)-type spores, or more generalized groups such as ascospores, and hyaline and colored basidiospores.

Data Handling

Fungal spore data were recorded directly into a Microsoft Access database designed specifically for this study to optimize efficiency of counting. Temperature (°C) and RH (%) data were collected at 10-minute intervals throughout the 24-hour sampling period using a LogBox-RHT datalogger (Audon Electronics, Chilwell, UK) and used to produce average values for each property. Occupants were asked to keep the windows closed and refrain from cleaning activities during the sampling. Open windows and sampler malfunction were among the criteria leading to the exclusion of 24 samples from analysis.

Outdoor Fungal Spore Concentrations

Outdoor air samples were collected using a 7-day recording volumetric spore trap (Burkard Manufacturing Co) located on the roof of a University of Leicester building, 1 km south of the city center, 12 m above the ground, and 60 m above sea level. We recently showed this system to be sufficient for aeroallergen analysis in a 41-km area [19]. Outdoor fungal spore concentrations were generated and analyzed as per indoor samples and time-matched to indoor sample periods.

Statistical Analysis

Samples were divided into seasons based on summer and winter solstices and vernal and autumnal equinoxes. Indoor and outdoor fungal spore counts were converted to mean fungal

spore concentration/m^3 air/day according to the correction factor specific to the microscope and magnification used. The correction factor was calculated using equation 1:

1) Mean fungal spore concentration/m^3 air/day = x(t/lsw), where x=raw data; t=total area of 24-hour trace (mm^2), l= length of trace (mm); s=volume of air sampled over the 24-hour period (m^3), and w=width of counting area (mm).

GraphPad Prism (Version 5) software (GraphPad Software, Inc., California, USA) was used to generate mean, median, minimum, maximum, and upper and lower quartile data for fungal spore and indoor/outdoor (I/O) ratios. To account for excess zeros, 1 was added to each data point to generate the I/O ratio (Y+1, where Y = fungal spore concentration/m^3 air/day). The correlation between indoor and outdoor concentrations was tested using Spearman's rank correlation coefficient.

The STATA (Version 10) data analysis and statistical software package (StataCorp, Texas, USA) was used to analyze the effect of season and home characteristics on fungal spore concentrations. Indoor and outdoor total fungal spore concentrations and abundant indoor fungi were log-transformed (Y+1) to normalize the data for parametric analyses. Initial assessments of seasonal variation were analyzed using the χ^2 test. Univariate linear regression was used to analyze the effect of home and season characteristics on indoor airborne fungal spore concentrations. Indoor fungal spore concentrations were then adjusted for outdoor levels and reanalyzed. Corresponding outdoor fungal spore concentrations were incorporated into multiple regression analyses of the effect of home and season characteristics on indoor fungal spore concentrations.

Zero-inflated negative binomial regression analyses were performed on nontransformed overdispersed, negatively skewed concentrations of fungal genera with excess zeros within the dataset. As with other indoor abundant fungi, χ^2 analyses were used to investigate seasonal variation.

Results

Airborne Fungal Spore Distributions

Indoor and outdoor fungal spore concentrations were highly variable and negatively skewed (Figure 1). Total airborne fungal spore concentrations were much lower indoors, ranging from 25 to 18 067 (median, 1135) in comparison to outdoor concentrations ranging from 539 to 237 144

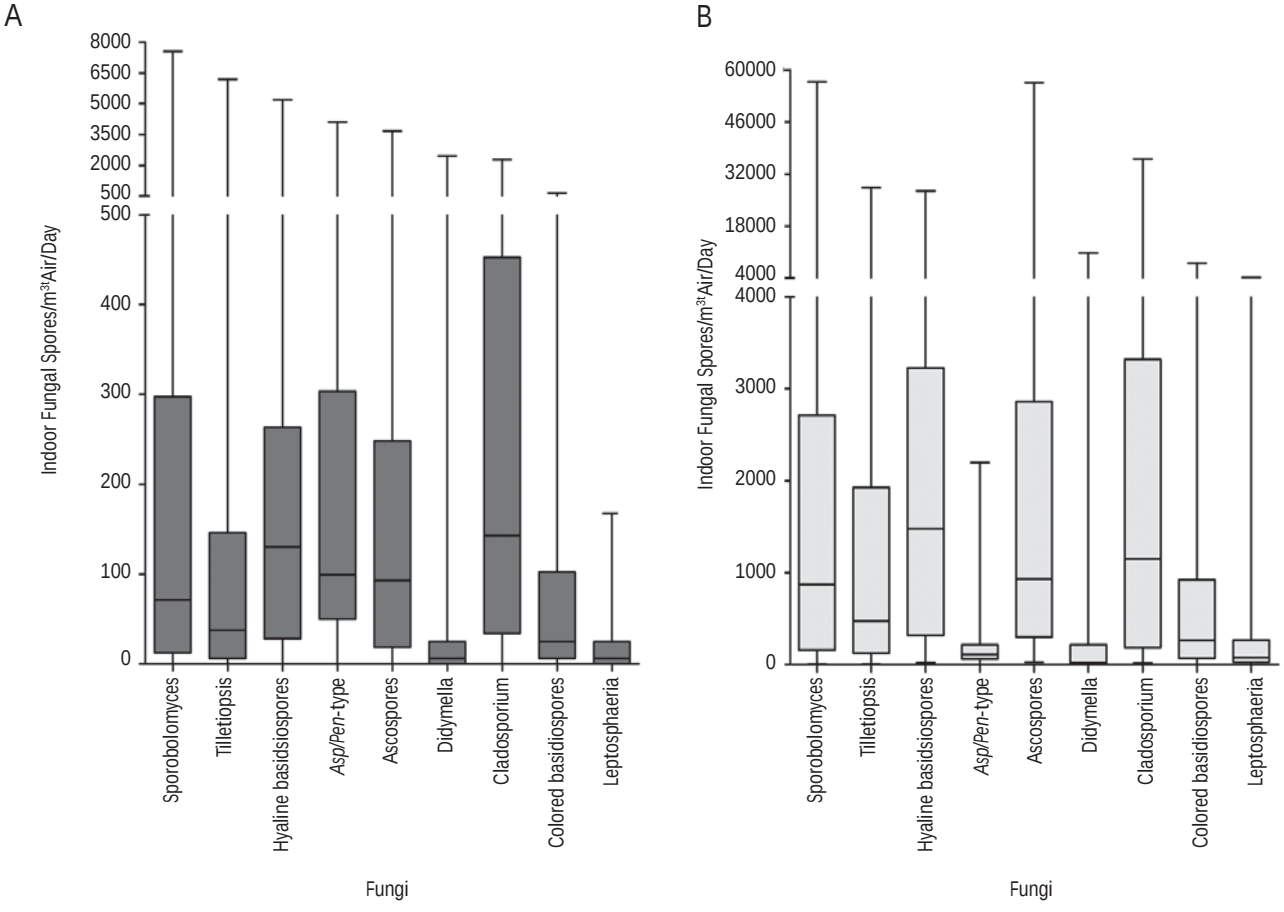

Figure 1. Airborne concentrations of abundant fungal genera indoors (A) and outdoors (B) (spores/m^3 air/day) based on 100 properties sampled.

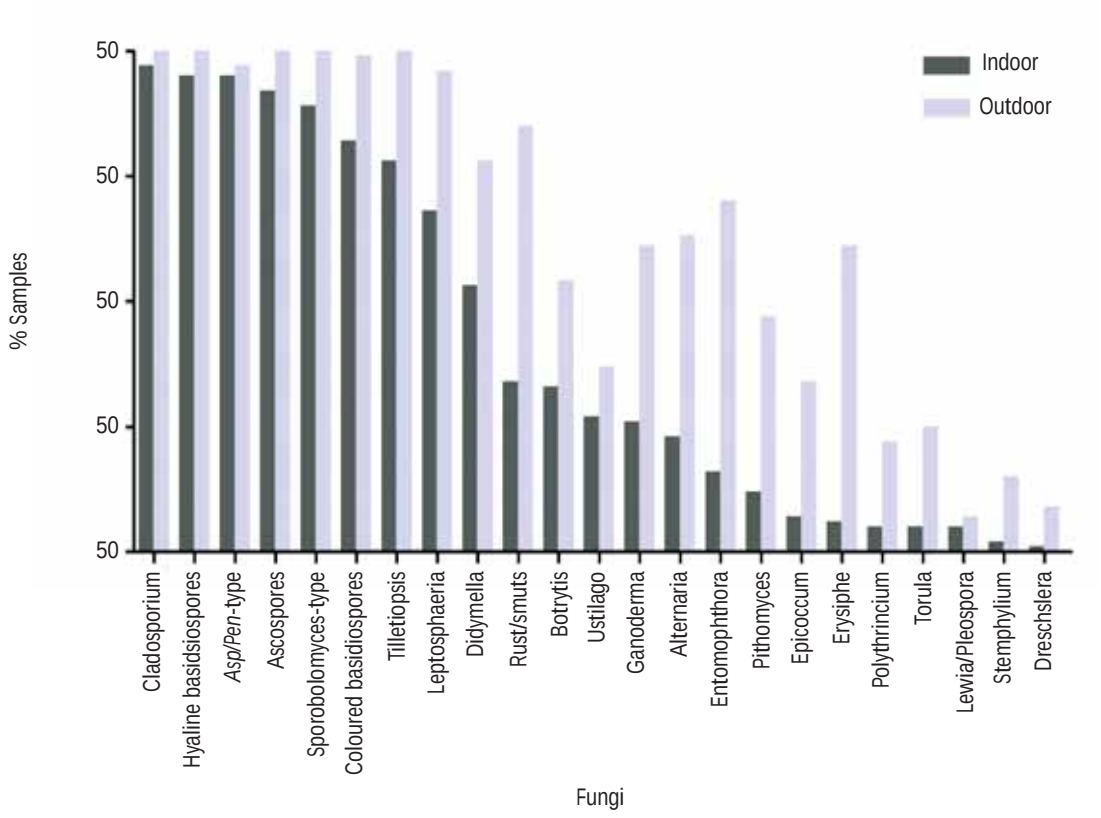

Figure 2. Percent of indoor and outdoor samples in which each of the fungal taxa was present, based on 100 properties sampled.

(median, 9201) spores/m^3 air/day. Indoor and outdoor fungal spore concentrations were positively correlated (r_s=0.5568, *P*<.0001).

Median indoor concentrations varied considerably between fungal taxa, ranging from 0 to 143 spores/m^3 air/day. Fungal taxa were easily divisible into abundant and low abundance categories based on their presence indoors. Abundant fungi were found in over 50% of samples (Figure 2) and had a median of >0, comprising *Sporobolomyces*, *Tilletiopsis*, hyaline basidiospores, *Asp/Pen*-type spores, ascospores, *Didymella*, *Cladosporium*, coloured basidiospores, and *Leptosphaeria* (Figure 1a). Hyaline basidiospores, colored basidiospores, and ascospores are highly generalized categories, where spore morphology is only possible to the level of phylum, enabling only very limited conclusions to be drawn from the data. Basidiospore and ascospore data were therefore excluded from further analyses. No further analyses were conducted on *Leptosphaeria* data since airborne concentrations were very low and never exceeded 167 spores/m^3 air/day.

Cladosporium spores were most frequently observed indoors (97% of properties), followed by hyaline basidiospores (95%) and *Asp/Pen*-type spores (95%, Figure 2). Fungal spore

Table 1. Distributions of Indoor/Outdoor Ratios of Total Fungal Spores and Abundant Indoor Taxa

Spore Type	Min	Max	Mean	SEM	Median	Q1	Q3
Cladosporium	0.002	8.908	0.339	0.093	0.173	0.078	0.280
Sporobolomyces	0.000	9.716	0.451	0.115	0.133	0.035	0.332
Asp/Pen-type	0.001	100.200	5.907	1.550	0.908	0.332	3.268
Tilletiopsis	0.000	5.537	0.334	0.072	0.099	0.017	0.305
Didymella	0.004	13.400	0.801	0.201	0.139	0.074	1.000
Total fungal spores	0.001	4.082	0.273	0.052	0.164	0.069	0.257

Abbreviations: *Asp/Pen, Aspergillus/Penicillium*; max, maximum; min, minimum; Q1, lower quartile; Q3, upper quartile.

concentrations were lower indoors than outdoors for all fungal taxa with the exception of *Asp/Pen*-type spores, which ranged from 0 to 4117 indoors and 0 to 2201 outdoors, suggesting indoor sources. *Asp/Pen*-type spores were thus described as indoor-predominant and removed from analyses of total fungal spore concentrations with housing characteristics. All other fungi were described as outdoor predominant where the primary source was outdoor. I/O ratios of total fungal spores and fungal taxa from the abundant group were highly variable and negatively skewed (Table 1). Outdoor predominant fungi had mean and median I/O ratios of <1, whilst *Asp/Pen*-type spores demonstrated a higher abundance indoors, with a mean I/O ratio of 5.907 and a median approaching 1 (Table 1).

Seasonal Variation

χ^2 analyses showed significant seasonal variations in indoor fungal spore concentrations of total fungal spores and outdoor abundant fungi. However, *Asp/Pen*-type spores did not vary significantly according to season (P=.563, Table 2). There was no significant variation in the I/O ratio for total fungal and abundant indoor fungi, with the exception of *Didymella* (P<.001).

Linear regression and zero-inflated negative binomial regression analyses (Tables 3 and 4, respectively) showed significantly higher indoor levels of *Cladosporium* and *Didymella* in the summer (P=.020 and P=.001, respectively), with lower levels of *Cladosporium* in the autumn (P=.017) and of *Didymella* in the winter (P=.019).

Housing Characteristics

Mean RH (%) had no significant effect on indoor fungal taxa with the exception of *Didymella* (P=.020, Table 4).

Table 2. Likelihood Ratio (χ^2) Analysis Comparing Absolute Levels of Indoor Fungal Spores, and the Ratio Between Indoor and Outdoor (I/O) Fungal Spore Concentrations, Over Different Seasons.

	Indoor	I/O ratio
	P[a]	P[a]
Cladosporium[b]	0.000	0.095
Sporobolomyces[b]	0.000	0.148
Asp/Pen-type[b]	0.563	0.351
Tilletiopsis[c]	0.015	0.767
Didymella[c]	0.000	0.000
Total fungal spores[b]	0.000	0.079

[a]Chi-square analysis. P<.05 indicates significant differences between the seasons.
[b]Linear regression analysis.
[c]Zero-inflated negative binomial regression analysis.

Decreased RH was predictive of the absence of *Tilletiopsis* and *Didymella* (P=.047 and P<.001 respectively, Table 4). Furthermore, mean temperature was negatively associated with the absence of *Didymella* (P=.015, Table 4).

Properties under 30 years old had significantly reduced levels of *Didymella* (P=.034, Table 4), properties 31 to 60 years old had the highest levels of *Tilletiopsis* (P=.024, Table 4) and the lowest levels of *Asp/Pen*-type spores (P=.028, Table 3), and properties over 90 years old had significantly elevated levels of *Asp/Pen*-type spores (P=.006, Table 3). Significantly reduced total fungal spores (P=.008), *Cladosporium* (P=.002), *Sporobolomyces* (P=.043), and *Didymella* (P=.019) were shown in flats in comparison to houses (Table 3 and 4). *Asp/Pen*-type spore concentrations were significantly higher

Table 3. Significant Associations Between Housing Characteristics and Indoor Fungal Spore Concentrations[a]

Housing Characteristics	No.	Indoor Fungal Spore Concentrations			
		Coef[b]	P	95% CI	
Flat	100	–0.409	.008	–0.707	–0.111
Asp/Pen-type					
31-60 years old	97	–0.380	.028	–0.716	–0.043
>90 years old	97	0.419	.006	0.125	0.712
Cavity wall insulation	88	–0.449	.002	–0.722	–0.176
Presence of a dog	100	–0.622	.005	–1.047	–0.197
Terraced	100	0.476	.003	0.161	0.792
Cladosporium					
Central heating	100	0.348	.029	0.037	0.658
Flat	100	–0.496	.002	–0.810	–0.182
Summer	100	0.321	.020	0.051	0.591
Autumn	100	–0.267	.017	–0.486	–0.048
Sporobolomyces					

Abbreviations: CI, confidence interval; Coef, regression coefficient; No., number of properties for which information was available.
[a]Spores/m^3 air/day; adjusted for outdoor concentrations.
[b]Linear regression analysis.

Table 4. Significant Associations Between Housing Characteristics and Indoor *Tilletiopsis* and *Didymella* Concentrations

Housing Characteristics		Indoor Fungal Spore Concentrations[a]				Absence Indoors[a]			
	No.	Coef[b]	*P*	95% CI		Coef[b]	*P*	95% CI	
Tilletiopsis									
Mean humidity	100	0.030	0.272	-0.024	0.084	-0.078	0.047	-0.155	-0.001
Semi-detached	100	0.936	0.015	0.180	1.691	0.575	0.513	-1.150	2.300
31-60 years old	97	0.995	0.024	0.128	1.862	-0.512	0.665	-2.828	1.803
Didymella									
Temperature	100	0.020	0.841	-0.179	0.220	-0.317	0.015	-0.572	-0.062
Mean humidity	100	0.047	0.020	0.007	0.086	-0.115	0.000	-0.177	-0.053
Presence of a dog	100	-0.823	0.037	-1.599	-0.048	-2.371	0.235	-6.284	1.542
Presence of a cat	100	0.730	0.030	0.069	1.392	0.561	0.238	-.371	1.493
Flat	100	-1.196	0.019	-2.194	-0.198	0.162	0.828	-1.306	1.631
Summer	100	0.907	0.001	0.357	1.457	-3.901	0.041	-7.646	-0.156
Winter	100	-1.251	0.019	-2.295	-0.207	1.418	0.020	0.227	2.610
0-30 years old	97	-0.794	0.034	-1.529	-0.059	0.707	0.154	-0.264	1.677

Abbreviations: CI, confidence interval; Coef, regression coefficient; No., number of properties for which information was available.
[a]Spores/m^3 air/day, adjusted for outdoor concentrations.
[b]Zero-inflated negative binomial regression analysis.

Table 5. Guideline Upper limits for Indoor Airborne Fungal Spore Concentrations Within Normal Ranges According to Season

Season	Indoor Fungal Spore Concentrations[a]					
	Total Fungal	*Sporobolomyces*	*Tilletiopsis*	*Asp/Pen*-type	*Didymella*	*Cladosporium*
Spring	2275	110	167	332	6	476
Summer	4520	856	219	116	287	854
Autumn	2443	253	127	321	6	208
Winter	1125	79	60	240	2	51

Abbreviations: *Asp/pen, Aspergillus/Penicillum.*
[a]Spores/m^3 air/day

in terraced properties (*P*=.003, Table 3), with *Tilletiopsis* concentrations highest in semidetached properties (*P*=.015, Table 4).

Central heating was positively associated with *Cladosporium* concentrations (P=.029, Table 3) whilst cavity wall insulation was negatively correlated with *Asp/Pen*-type spores (*P*=.002, Table 3).

The presence of a dog was negatively associated with indoor levels of *Asp/Pen*-type spores (*P*=.005, Table 3) and *Didymella* spores (*P*=.037, Table 4), whilst that of a cat was positively associated with *Didymella* concentrations (*P*=.030, Table 4).

No significant effect of floor covering, presence of damp, visible mold, condensation, or double glazing was shown on the levels of any fungal taxa.

Typical Ranges of Indoor Airborne Fungal Spores

Upper quartile ranges of seasonal distributions in abundant indoor taxa were used to provide guidelines for typical indoor fungal spore concentrations in noncomplaint properties (Table 5).

Discussion

Indoor Fungal Spore Distributions

Indoor fungal spore concentrations are highly variable and follow nonnormal distributions which are negatively skewed [20], as shown by our data. Total indoor fungal spore concentrations ranged considerably from 25 to 18 067 spores/m^3 air/day, exhibiting higher variability than reported previously [21].

Abundant indoor fungi typically comprised *Cladosporium, Asp/Pen*-type spores, *Sporobolomyces, Didymella*, ascospores, hyaline and coloured basidiospores, and *Leptosphaeria*. Fungi capable of colonization of indoor substrates may pose a risk to health or cause building degeneration, requiring expensive remediation strategies. *Cladosporium, Aspergillus*, and *Penicillium* are common indoor and outdoor fungal genera and are known contaminants of building materials. *Cladosporium* species are important aeroallergens with growth potentiated on water-damaged substrates [22], although levels were not significant in our study, possibly due to our focus on noncomplaint properties. *Aspergillus* and *Penicillium*

are indoor-predominant fungi, colonizing a wide variety of substrates including fabric and paper [23,24], and can be concealed within wall cavities or building materials [9]. *Asp/Pen*-type spores are consistently present outdoors in low concentrations, and indoor concentrations can easily match or even exceed outdoor levels, independently of season and outdoor concentrations. In contrast, most other fungi are only found indoors due to passive movement from outside, and indoor concentrations of these fungi are probably related to outdoor fungal concentrations, air flow, and ventilation rates.

Indoor fungal spore concentrations are typically much lower than outdoor concentrations, unless an indoor source is present [14]. Based on the median I/O ratio for total fungal spores, our data show that indoor concentrations represent approximately 16% of outdoor levels, although the contribution from outdoors may be as high as 26% (based on the upper quartile limit for total fungal spores).

Seasonal Variation

Seasonal variation in indoor airborne fungal spore concentrations has been well documented; however, whilst absolute values fluctuate according to season, the relative proportion of spores moving indoors from outdoors remains unchanged [16]. We only observed a significant effect of season on the I/O ratio in our study for *Didymella*, which can probably be explained by stark contrasts in daily fluctuations of *Didymella*, possibly due to sudden sporadic bursts in the release of *Didymella*, triggered by rainfall in the summer [25]. *Didymella* spores are primarily released during summer months, when concentrations can reach peaks of 5000 spores/m^3 air/day and occasionally exceed 40 000 spores/m^3 air/day, before rapidly diminishing on subsequent days (unpublished data).

Cladosporium and *Didymella* demonstrated significant seasonal relationships, both being higher in the summer, when they reach outdoor peaks. Increased indoor RH has been associated with elevated levels of fungal spores and respiratory symptoms [10,15,22]. However, none of the properties sampled in our study reached levels of 80% RH, which would be expected to encourage fungal contamination [7]. The positive association between RH and *Cladosporium*, *Sporobolomyces*, and *Tilletiopsis* was lost after adjusting for outdoor levels, suggesting that previous associations with RH may have been misleading, with outdoor factors playing a key role. This supports reports from a previous study that the positive association between RH and indoor fungal spore levels was lost following adjustment for outdoor concentrations [22].

Influence of Home Characteristics on Outdoor-Predominant Indoor Fungal Spores

Increased temperature has been associated with elevated indoor fungal spore levels [10]; however, this was not shown by our data after adjustment for outdoor data. As with humidity, previous associations with mean temperature may be explained by increasing outdoor concentrations and infiltration indoors during the warmer summer months. After outdoor correction, only *Didymella* was significantly affected by temperature, with mean temperature being negatively associated with the absence of *Didymella*, a pathogen of vegetation which is diminished during the winter months when temperatures are reduced.

Indoor fungal spore concentrations of outdoor-predominant fungi were lower in flats than houses, as previously described [15], which may be explained by reduced infiltration or carriage of spores indoors. Elevated dust-borne fungal concentrations have been shown in carpet [15,26] but we found no association between floor covering and airborne fungal levels. This highlights the problem with the comparative analysis of dust and air samples since elevated levels of dust-borne mold concentrations do not automatically indicate increased airborne levels [15].

Increased dust-borne levels of ß(1→3) glucans (a major cell wall component of fungi and yeasts) have been shown in houses with central heating (and built after 1970) [27]. We showed a positive association between central heating and *Cladosporium* levels, which can be elevated in areas of condensation. It is possible that the location of radiators in centrally heated houses, typically beneath windows, would encourage the release of *Cladosporium* spores from window frames; however, we found no associations between *Cladosporium* levels and condensation reports or use of heating during sampling.

Influence of Home Characteristics on Fungal Spores With Potential Indoor Sources

Age and type of housing are important predictors of indoor-predominant fungal spore concentrations, with higher concentrations of *Asp/Pen*-type spores in old, terraced properties and reduced *Asp/Pen*-type spore levels in properties 31 to 60 years old. This indicates that differences in building design and materials are important determinants of indoor levels. The negative association between *Asp/Pen*-type spore concentrations and the presence of dogs within properties is also probably explained by housing age and type, since there were no dogs in the old terraced properties sampled (data not shown).

Installation of double glazing and central heating has been associated with increased RH, a decrease in thermotolerant *Aspergilli*, and an increase in *Aspergillus fumigatus*, a clinically relevant fungus with a higher water affinity (0.98-0.99) [28]. Whilst we detected no association between indoor fungal spore concentrations and double glazing, the presence of cavity wall insulation was negatively correlated with *Asp/Pen*-type spore concentrations. Cavity wall insulation would be expected to add to the effects of central heating and double glazing. Therefore, a negative association between *Asp/Pen*-type spores and the presence of cavity wall insulation may be explained by species of *Aspergillus* or *Penicillium* within these properties having lower water affinities. Species-specific analysis of indoor air samples would be required to investigate this further. There is considerable variation in the prevalence and pathogenicity of *Aspergillus* and *Penicillium* species; therefore, properties determined as having elevated levels of *Asp/Pen*-type spores will require further species-specific analysis to determine whether predominating species have known adverse health effects.

Establishing Normal Ranges

Sampling inside and outside simultaneously is the gold standard for investigating indoor spore levels. Unfortunately,

this is not always feasible. The upper quartile value for each type of fungi in a given season from this study can be used as a guide in the absence of outdoor data. Properties with fungal spore concentrations exceeding upper baseline limits may warrant further investigation, whilst lower concentrations can be assumed to be within normal ranges.

In conclusion, this study provides the first set of typical indoor ranges of abundant indoor fungi reported in the UK, which can be used for comparative purposes in future studies of fungal exposure. Previous studies have been conducted in different countries and climatic regions or commercial properties where differences in fungal flora, climate, and building design and materials almost certainly affect indoor levels [12,29]. Indoor fungal spore concentrations are highly variable and inconsistent between properties, even those of similar type and age. Fungi vary in growth and sporulation patterns, and require different conditions for release, making collective modeling difficult. However, approximately 16% of outdoor airborne fungal spore concentrations can infiltrate indoors. If outdoor data is available, properties with I/O ratios exceeding 16% may warrant further investigation. This is certainly the case for I/O ratios exceeding 26%. Air sample data should be interpreted with caution as levels exceeding guideline ranges are by no means a definitive indication of the presence of an indoor source. Infiltration from outdoors during transient periods, increased occupancy or activity, cleaning, refurbishment and remediation works may influence indoor airborne fungal spore concentrations, and must be taken into account in analyses.

Within noncomplaint properties where no indoor source is evident, indoor concentrations of individual spore types typically reflect outdoor concentrations and seasonal patterns. Interestingly, we have shown lower levels of outdoor-predominant fungi in flats, with building design, type, and age having a significant effect on the infiltration of fungal spores. *Asp/Pen*-type spores are indoor-predominant fungi with the highest concentrations found in old, noninsulated, terraced properties.

Acknowledgments

The authors would like to thank the Midlands Asthma and Allergy Research Association (MAARA) for providing funding for the study, and the 124 volunteers who agreed to have their homes sampled.

Previous Presentation: Preliminary data from this study was presented at the British Society for Allergy and Clinical Immunology Annual Meeting 2007.

References

1. Chapman MD. Challenges associated with indoor moulds: Health effects, immune response and exposure assessment. Med Mycol. 2006;44:S29-S32.
2. Kurup VP, Shen HD, Banerjee B. Respiratory fungal allergy. Microbes Infect. 2000;2:1101-10.
3. Burge HA. An update on pollen and fungal spore aerobiology. J Allergy Clin Immunol. 2002;110:544-52.
4. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. Eur Respir J. 2006 Mar;27:615-26.
5. Platts-Mills TAE. Is there a dose-response relationship between exposure to indoor allergens and symptoms of asthma? J Allergy Clin Immunol. 1995;96:435-40.
6. D'Amato G, Liccardi G, D'Amato M, Holgate S. Environmental risk factors and allergic bronchial asthma. Clin Exp Allergy. 2005;35:1113-24.
7. Gravesen S. Microbiology on indoor air '99 - what is new and interesting? An overview of selected papers presented in Edinburgh, August, 1999. Indoor Air. 2000;10:74-80.
8. Dales RE, Miller D, McMullen E. Indoor air quality and health: Validity and determinants of reported home dampness and moulds. Int J Epidemiol. 1997 Feb;26:120-5.
9. Morey PR, Hull MC, Andrew M. El Niño water leaks identify rooms with concealed mould growth and degraded indoor air quality. Int Biodeterior Biodegrad. 2003;52:197-202.
10. Ren P, Jankun TM, Belanger K, Bracken MB, Leaderer BP. The relation between fungal propagules in indoor air and home characteristics. Allergy. 2001;56:419-24.
11. Verhoeff AP, Burge HA. Health risk assessment of fungi in home environments. Ann Allergy Asthma Immunol. 1997;78:544-54.
12. Codina R, Fox RW, Lockey RF, DeMarco P, Bagg A. Typical levels of airborne fungal spores in houses without obvious moisture problems during a rainy season in Florida, USA. J Investig Allergol Clin Immunol. 2008;18:156-62.
13. Sterling M, Rogers C, Levetin E. An evaluation of two methods used for microscopic analysis of airborne fungal spore concentrations from the Burkard Spore Trap. Aerobiologia. 1999;15:9-18.
14. Stern MA, Allitt U, Corden J, Millington W. The investigation of fungal spores in intramural air using a Burkard continuous recording air sampler. Indoor Built Environ. 1999;8:40-8.
15. Chew GL, Rogers C, Burge HA, Muilenberg ML, Gold DR. Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics. Allergy. 2003;58:13-20.
16. Shelton BG, Kirkland KH, Flanders WD, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl Environ Microbiol. 2002 Apr;68:1743-53.
17. Baxter DM, Perkins JL, McGhee CR, Seltzer JM. A regional comparison of mold spore concentrations outdoors and inside "clean" and "mold contaminated" southern California buildings. J Occup Environ Hyg. 2005 Jan;2:8-18.
18. Corden J, Millington W. The long-term trends and seasonal variation of the aeroallergen Alternaria in Derby, UK. Aerobiologia. 2001;17:127-36.
19. Pashley CH, Fairs A, Edwards RE, Bailey JP, Corden JM, Wardlaw AJ. Reproducibility between counts of airborne allergenic pollen from two cities in the East Midlands, UK. Aerobiologia. 2009;25:249-63.
20. Quezada NV, Lange JH. Final clearance criteria after mould remediation. Indoor Built Environ. 2004 Jun;13:199-203.
21. Gots RE, Layton NJ, Pirages SW. Indoor health: Background levels of fungi. AIHA J. 2003 Jul-Aug;64:427-38.

22. Garrett MH, Rayment PR, Hooper MA, Abramson MJ, Hooper BM. Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children. Clin Exp Allergy. 1998;28:459-67.
23. Emberlin J, Newman T, Bryant RH. The incidence of fungal spores in the ambient air and inside homes: Evidence from London. Aerobiologia. 1995;11:253-8.
24. Samson RA, Houbraken J, Summerbell RC, Flannigan B, Miller JD. Common and important species of fungi and actinomycetes in indoor environments. In: Flannigan B, Samson RA, Miller JD, editors. Microorganisms in home and indoor work environments. London: Taylor & Francis; 2001. p. 287-474.
25. Corden JM, Millington WM. Didymella ascospores in Derby. Grana. 1994;33:104-7.
26. Dharmage S, Bailey M, Raven J, Mitakakis T, Thien F, Forbes A, Guest D, Abramson M, Walters EH. Prevalence and residential determinants of fungi within homes in Melbourne, Australia. Clin Exp Allergy. 1999;29:1481-9.
27. Douwes J, Doekes G, Heinrich J, Koch A, Bischof W, Brunekreef B. Endotoxin and beta(1→3)-glucan in house dust and the relation with home characteristics: A pilot study in 25 German houses. Indoor Air. 1998;8:255-63.
28. Hirsch T, Hering M, Burkner K, Hirsch D, Leupold W, Kerkmann ML, Kuhlisch E, Jatzwauk L. House-dust-mite allergen concentrations (Der f 1) and mold spores in apartment bedrooms before and after installation of insulated windows and central heating systems. Allergy. 2000 Jan;55:79-83.
29. MacIntosh DL, Brightman HS, Baker BJ, Myatt TA, Stewart JH, McCarthy JF. Airborne fungal spores in a cross-sectional study of office buildings. J Occup Environ Hyg. 2006;3:379-89.

Manuscript received December 17, 2009; accepted for publication February 27, 2010.

Catherine H Pashley

Aerobiology Unit, Institute for Lung Health
Department of Infection, Immunity and Inflammation
University of Leicester, Leicester, UK
E-mail: chp5@le.ac.uk