

Airborne fungal spores in 80 homes in the Latrobe Valley, Australia: levels, seasonality and indoor-outdoor relationship

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Abstract

Airborne viable and total fungal spores were sampled inside and outside 80 houses in the Latrobe Valley, Victoria, Australia as part of a larger indoor environmental study. Each residence was visited six times over a period of 1 year for sample collection, and fungal spore samples were collected from at least three indoor sites and from an outdoor site. Viable spores were sampled using an Andersen sampler, while total spores were assessed using a Burkard spore trap. Identification of fungal colonies to genera level was performed in two seasons; winter and late spring. The most common fungal genera/groups were *Cladosporium*, *Penicillium*, and yeasts, both indoors and outdoors in winter and late spring. Outdoor levels were higher than those indoors throughout the year, and a significant seasonal variation in spore levels was seen both indoors and outdoors with overall maxima in summer. Contrary to this trend, the levels of *Aspergillus*, yeasts, *Cephalosporium* and *Gliocladium* were higher in winter. Most fungal genera were found in greater concentrations outdoors compared to indoors, but *Penicillium* was more common indoors. Outdoor spore levels were a significant influence on indoor levels, but seasonal differences suggest that other influences are important. © 1997 Elsevier Science Ireland Ltd.

Keywords: Fungal spores; Indoor air; Viable spores; Total spores; Outdoor influence; Australia

1. Introduction

Indoor exposure to fungi has been suggested as a major health risk, which can cause a significant increase in the risk of respiratory symptoms and allergy among residents (Miller, 1992). In Australia, asthma prevalence among children is high (Robertson et al., 1993), and the reasons for this are not understood, but exposure to indoor contaminants has been suggested as a possible contributing factor. Previously, little data on exposure levels for various indoor contaminants in Australia were available, and a need to define the exposure to indoor contaminants in Australian dwellings was apparent. Available data suggested that the indoor environment in houses in south-eastern Aus-

tralia is conducive to house dust mite contamination (Tovey, 1993) and possibly also to fungal contamination (Godish et al., 1993). In order to clarify whether this is the case, this paper presents data from the fungal portion of a larger indoor environmental study which also included measurements of house dust mite allergen, nitrogen dioxide, and formaldehyde. An outline of the methodology for the study has been published (Garrett et al., 1995).

2. Materials and methods

Eighty households in the Latrobe Valley, Victoria, Australia were recruited for the study. All households had at least one child between 7 and 14 years of age, and 43 of the households had at least one asthmatic child, while the remaining 37 households had only

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non-asthmatic children (results related to health outcomes will be discussed in a separate paper). Sample collection was carried out during six visits performed on a 2 monthly cycle to the households, spanning 1 year from March 1994 until February 1995. Both viable (culturable) fungal spores (propagules) and total fungal spores were assessed, and samples were collected in bedrooms, living room, kitchen and outside each house. Indoor viable and total spore samples were taken at a height of 0.5–1.0 m above floor level, while outdoor samples were taken near ground level close to the house in order to estimate the local air spora. Indoor samples were collected under conditions of normal room use, and no attempt to reduce ventilation prior to sample collection was made.

For viable spore sampling, a single-stage Andersen impactor (Andersen, Atlanta, GA) was used, and it was operated for 2 min at a flow rate of 24 l/min. Malt extract broth (Oxoid), solidified with technical agar no. 3 (Oxoid) was used as the collection media, and samples were incubated in the dark for 4–5 days before colonies were counted. The positive hole conversion method (Andersen, 1958) was applied to the results which are expressed as colony forming units per cubic meter of air (CFU/m³). During two of the six sampling periods, winter (July) and late spring (November), colonies were identified to genera level. Colonies which were not sporulating on malt extract agar were transferred to oat meal agar and V8 media (Stevens, 1974), and incubated under ultraviolet light to induce sporulation. Isolates which still failed to sporulate were not identified and are referred to as un-identified. Colonies which were overgrown on the sample plate and could not be identified are referred to as un-known isolates.

Total fungal spores were sampled using a Burkard personal spore trap (Burkard, Rickmansworth, Hertfordshire, England). The sampler was operated for 9 min at a flow rate of 10 l/min. Sampling sites were the same as for viable sampling, but kitchen samples were omitted except for the first sampling period due to a limited battery life of the sampler. Microscope slides coated with a thin layer of silicone grease were used for collection of particles, and Kaiser's glycerol jelly with added aniline blue solution was used as the mounting media. Cover slips were sealed on using Estapol (Wattyl, Australia). Spores were counted using a light microscope at a magnification $\times 1000$, and a total of ten sweeps across the deposition area were counted, equalling 5% of the total area. Results are expressed as number of spores per cubic meter of air (spores/m³).

Spore concentration data were positively skewed, and non-parametric tests such as Mann–Whitney U were therefore used for comparisons, while a log₁₀-transformation was applied to gain a close-to-normal distribution for calculation of correlation coefficients. All statistical analyses were performed using SPSS for Win-

dows, version 6.0, and reported comparisons and correlations were considered significant when $P < 0.05$.

3. Results

The median indoor viable mould spore concentration over the year of sampling was 812 CFU/m³ based on individual samples, with a range from < 20 to 54 749 CFU/m³. Outdoor levels ranged from 21 to 18 396 CFU/m³ with a median of 1042 CFU/m³. Indoor levels were lower than outdoor levels (Fig. 1), and the difference was significant for all periods, except in summer (January–February, $P = 0.23$). A seasonal influence on spore levels is apparent, both indoors and outdoors, with indoor medians ranging from a low of 479 CFU/m³ in early spring (September) to a high of 1583 CFU/m³ in summer (January–February). A histogram showing the distribution of the mean indoor viable fungal spore levels in winter (based on sampling in different rooms) is presented in Fig. 2. The average indoor spore count was less than 300 CFU/m³ in 15% of houses, while 85% of houses had a level lower than 900 CFU/m³.

The median indoor level of total spores over the year of sample collection was 7778 spores/m³, with a range from 667 to 118 222 spores/m³. Outdoor levels ranged from 222 to 163 778 spores/m³, with a median of 8889 spores/m³. Indoor medians were significantly lower than outdoors for all sampling periods (Fig. 3), except in summer (January–February, $P = 0.15$), and in early spring (September) indoor levels actually exceeded those outdoors, but not significantly ($P = 0.45$). Signifi-

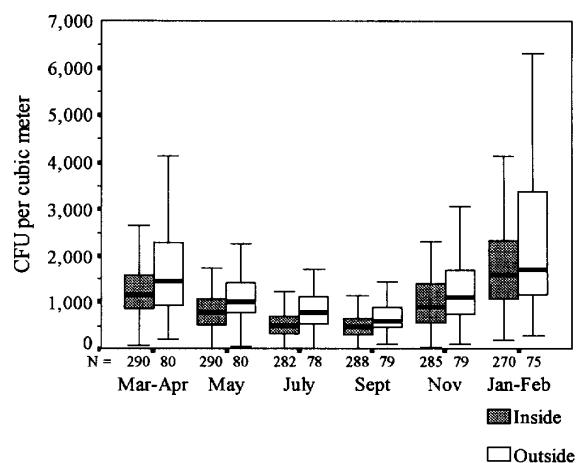


Fig. 1. Viable mould spore concentrations (CFU/m³) by location and sampling period. Box-plot shows median, with 25–75th percentiles as the box limits. Whiskers extend to the furthest point which is not an outlier (defined as > 1.5 interquartile ranges from the end of the box).

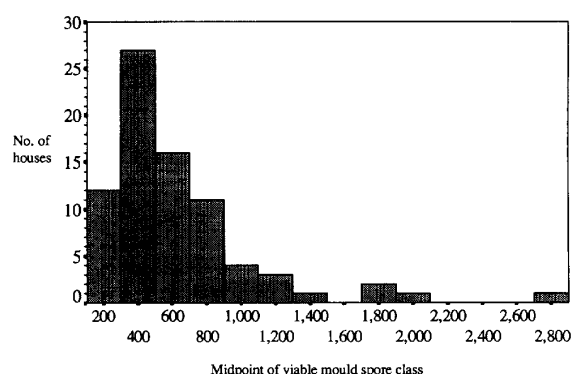


Fig. 2. Distribution of mean indoor viable spore counts (CFU/m³) in winter (July), based on sampling in different rooms, $n = 78$ houses.

cant seasonal variation is apparent, and indoor medians ranged from a low of 5111 spores/m³ in early spring (September) to a high of 12 778 spores/m³ in early autumn (March–April).

Identification of viable fungal colonies to genera level was performed in winter (July) and late spring (November). The most common genera both indoors and outdoors were *Cladosporium*, *Penicillium* and yeasts in both sampling periods. Identified genera in winter are listed in Table 1, and late spring data are presented in Table 2. In general, indoor counts were higher in November compared to July ($P < 0.0001$). Similarly, significantly higher indoor levels of *Cladosporium*, and *Botrytis* were seen in late spring, while certain genera were significantly more common in winter: yeasts, *Aspergillus*, *Acremonium*, and *Gliocladium*.

Some significant differences in the concentration of specific genera/groups indoors compared to outdoors

Table 1
Fungal genera/groups in winter (July).

	Indoors		Outdoors	
	% colonies	% houses	% colonies	% houses
<i>Cladosporium</i>	42.3	100.0	46.5	96.1
<i>Penicillium</i>	16.8	100.0	10.2	82.0
Yeast	14.0	100.0	13.0	85.9
<i>Aspergillus</i>	2.6	73.1	1.9	42.3
<i>Acremonium</i>	2.2	84.6	1.9	47.4
<i>Botrytis</i>	1.1	44.9	1.4	38.5
<i>Epicoccum</i>	0.9	53.8	1.0	30.8
<i>Spicaria</i>	0.8	5.1	0.1	3.8
<i>Papularia</i>	0.5	30.8	0.3	9.0
<i>Alternaria</i>	0.4	19.2	0.3	10.3
<i>Isaria</i>	0.3	14.1	0.1	3.8
<i>Monocillium</i>	0.2	10.3	0.2	6.4
<i>Gliocladium</i>	0.2	15.4	0.1	3.8
<i>Candida</i>	0.2	14.1	—	—
<i>Phoma</i>	0.2	12.8	0.2	5.1
<i>Gilmaniella</i>	0.1	x	—	—
<i>Oidium</i>	0.1	7.7	<0.1	x
<i>Verticillium</i>	0.1	3.8	—	—
<i>Rhizopus</i>	0.1	4.8	—	—
<i>Phomopsis</i>	0.1	5.1	0.1	2.6
<i>Chaetomium</i>	0.1	3.8	<0.1	x
<i>Bipolaris</i>	0.1	5.1	—	—
<i>Mucor</i>	<0.1	3.8	0.1	3.8
<i>Geotrichum</i>	<0.1	3.8	0.1	2.6
<i>Fusarium</i>	<0.1	x	—	—
<i>Scopulariopsis</i>	<0.1	x	—	—
<i>Cylindrocarpum</i>	<0.1	2.6	—	—
<i>Ulocladium</i>	<0.1	x	—	—
<i>Colletotrichum</i>	<0.1	x	—	—
<i>Chrysosporium</i>	<0.1	x	—	—
<i>Basipetospira</i>	<0.1	x	—	—
<i>Phialophora</i>	—	—	<0.1	x
<i>Glomerularia</i>	—	—	<0.1	x
Unidentified ^a	7.1	—	8.4	—
Unknown ^b	9.6	—	14.0	—

x Only encountered in one house.

^a A number of genera which were isolated and cultured, but failed to sporulate and were not identified.

^b Colonies which were overgrown or not successfully transferred.

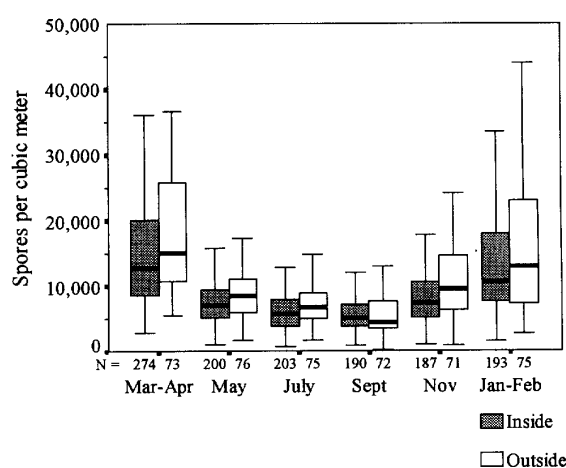


Fig. 3. Total mould spore levels (spores/m³) by location and sampling period. See Fig. 1 for definitions of box plot components.

were noted. Significantly higher outdoor levels were seen for *Cladosporium*, *Alternaria*, *Botrytis*, and *Epicoccum*. The same behaviour was observed for yeasts, but the difference was not significant ($P < 0.1$). Only *Penicillium* was significantly more common indoors.

An influence of outdoor spore concentrations on indoor levels was evaluated by linear regression of log₁₀-transformed data, using indoor levels as the dependent variable. Average indoor and outdoor results for each sampling period were used, and a separate correlation coefficient was calculated for viable and total spore levels. Coefficients of determination (R^2) are presented in Table 3.

4. Discussion

The method used for determination of airborne fungal spores has a major influence on the results (Miller, 1992), and wide variations in spore levels are the rule rather than the exception, even between samples from the same house. Therefore, comparisons with previous literature must be done with caution, but despite limitations, a comparison with spore levels and genera recorded in other countries can provide some insight into the differences and similarities in aerobiology.

The levels of viable fungal spores recorded in the present study are somewhat higher than results from Great Britain and the Netherlands, which can be regarded as comparable climate areas (Hunter et al., 1988; Verhoeff et al., 1992). Hunter et al. (1988) reports that around one third of houses in Great Britain recorded viable spore levels below 200 CFU/m³ in winter, while only 15% of houses in the present study

Table 2
Fungal genera/groups in late spring (November)

	Indoors		Outdoors	
	% colonies	% houses	% colonies	% houses
<i>Cladosporium</i>	61.5	100.0	68.9	100.0
<i>Penicillium</i>	19.2	97.3	4.5	66.7
Yeast	4.5	100.0	4.5	78.7
<i>Botrytis</i>	1.0	64.0	0.8	26.7
<i>Acromonium</i>	0.8	66.7	0.5	22.7
<i>Epicoecum</i>	0.5	56.0	0.9	37.3
<i>Alternaria</i>	0.3	37.3	0.6	21.3
<i>Aspergillus</i>	0.2	18.7	0.1	5.3
<i>Papularia</i>	0.2	29.3	0.2	10.7
<i>Phoma</i>	0.1	14.7	0.1	4.0
<i>Mucor</i>	0.1	12.0	<0.1	x
<i>Gliocladium</i>	<0.1	8.0	<0.1	2.7
<i>Monocillium</i>	<0.1	5.3	<0.1	x
<i>Candida</i>	<0.1	6.7	<0.1	x
<i>Isaria</i>	<0.1	2.7	0.1	2.7
<i>Fusarium</i>	<0.1	2.7	0.1	6.7
<i>Ulocladium</i>	<0.1	4.0	—	—
<i>Glomerularia</i>	<0.1	4.0	—	—
<i>Trichoderma</i>	<0.1	2.7	<0.1	2.7
<i>Chaetomium</i>	<0.1	2.7	—	—
<i>Rhizopus</i>	<0.1	2.7	<0.1	x
<i>Stemphylium</i>	<0.1	x	<0.1	x
<i>Beauveria</i>	<0.1	x	—	—
<i>Geotrichum</i>	<0.1	x	—	—
<i>Diplococcium</i>	<0.1	x	—	—
<i>Monilia</i>	<0.1	x	—	—
<i>Verticillium</i>	<0.1	x	—	—
<i>Spicaria</i>	—	—	1.2	x
<i>Bipolaris</i>	—	—	<0.1	x
Unidentified ^a	2.4	—	2.6	—
Unknown ^b	7.6	—	11.6	—

x Only encountered in one house.

^a A number of genera which were isolated and cultured, but failed to sporulate and were not identified.

^b Colonies which were overgrown or not successfully transferred.

Table 3

Coefficients of determination (R^2), calculated from linear regression with indoor spore levels as dependent variable and outdoor spore levels as independent variable, in six sampling periods ($\log_{10}$ -transformed CFU/m³ or spores/m³ values)

Sampling period	Viable spores	Total spores
March–April	0.24*	0.46*
May	0.12*	0.14*
July	0.25*	0.23*
September	0.01	0.11*
November	0.07**	0.03
January–February	0.46*	0.68*

$n = 80$ houses.

* $P < 0.01$, ** $P < 0.05$.

had winter levels below 300 CFU/m³. From the Netherlands, Verhoeff et al. (1992) report viable spore levels just over 800 CFU/m³ (geometric mean) indoors in spring which is somewhat lower than spring levels with a geometric mean of 890 CFU/m³ recorded in the present study. A previous study conducted in the Latrobe Valley, Victoria reports a geometric mean of 495 CFU/m³ indoors in winter (Godish et al., 1996), which is very similar to the geometric mean of 502 CFU/m³ recorded in the present study. In a subtropical climate such as Taiwan, an indoor geometric mean of 1950 CFU/m³ was recorded in Tai-Chi, while 1288 CFU/m³ is reported from Taipei in summer (May–July), (Li and Kuo, 1994). Interestingly, these levels are similar to the 1593 CFU/m³ (geometric mean) recorded in the present study during summer, despite a much cooler and drier climate in this part of Australia compared to Taiwan. It would appear that the viable spore levels recorded in this study are among the highest reported.

Total spore levels are not reported as frequently as viable spore levels, but a previous study in the Latrobe Valley, Victoria recorded levels with a geometric mean of 10 488 spores/m³ during winter (Godish et al., 1996). This is somewhat higher than the results from the present study where a median of around 6000 spores/m³ was recorded in winter. It is possible that the difference can be accounted for by seasonal variation or by a difference in sampling technique. Godish et al. (1996) reduced natural ventilation prior to sample collection, and this would have increased indoor levels if indoor sources were present. The proportion of total spores which were found to be viable was about 10% in the present study, which is similar to previous reports (Horner et al., 1994; Godish et al., 1996).

The present study shows that *Cladosporium*, *Penicillium* and yeasts are the most common fungal genera/groups in south-eastern Australia, encountered in 100% of houses. These results confirm previous reports of remarkable similarities between climate regions as to the most commonly isolated fungal genera (Pasanen,

1992). Other common genera included *Aspergillus*, *Botrytis*, *Acremonium*, and *Epicoccum* which were encountered in more than 50% of houses (Tables 1 and 2), but each only accounted for less than 2% of indoor colonies. All above mentioned genera have been listed among the most common in at least one previous indoor air study (Hunter et al., 1988; Verhoeff et al., 1992; Beguin and Nolard, 1994), but a common occurrence of *Botrytis*, *Acremonium* and *Epicoccum* does not tend to be reported universally. It is worth noting that un-known and un-identified isolates account for as much as 17% of total indoor colonies in winter, and 10% of indoor colonies in late spring. In many previous studies, such isolates have been referred to as *mycelia sterilia*. It is likely that a large proportion of these colonies are species belonging to the fungal groups basidiomycetes or ascomycetes as these are often not easily cultured using conventional air survey techniques (Horner et al., 1994). It is even likely that such spores fail to grow on malt extract altogether, thus leading to a serious underestimation. Consequently, such spores may be of much greater importance than can be determined from the present results. Total spore samples can be used to give an estimate of their abundance, but no attempt was made to identify spores on total spore samples in this study.

Seasonal variation in ambient airborne spore levels has been documented for Australian conditions previously (Derrick and McLennan, 1963), and the present study confirms a reduction in total levels during winter with a magnitude of variation from winter to summer around three times for both viable and total spore levels. However, despite significant variation, a substantial concentration remains throughout the year, contrary to cooler climates where snow cover renders outdoor levels negligible (Miller et al., 1988). Consequently, it is possible that exposure to fungal spores in south-eastern Australia is high enough to cause allergic reactions and other health effects in some individuals throughout the year.

Differences in the concentration for certain genera were seen between the two seasons in which colonies were identified; winter and late spring. In accordance with the literature, *Cladosporium* concentrations were significantly higher in the warmer season (Simeray et al., 1993), as was *Botrytis* (Ebner et al., 1989). On the other hand, yeasts, *Acremonium*, and *Aspergillus* were more common indoors in winter, following the trends suggested by Ebner et al. (1989), while *Gliocladium* was also more common in winter, a genus for which there appears to be no previous reports of seasonality.

Certain fungal genera, such as *Penicillium* and *Aspergillus* are commonly referred to as 'indoor fungi' due to their greater relative prevalence indoors compared to outdoors in most homes (Solomon, 1976). Indeed, *Penicillium* was significantly more common indoors

compared to outdoors in the present study, but no significant differences between environments were seen for *Aspergillus*. On the other hand, *Cladosporium*, yeasts, *Alternaria*, *Botrytis* and *Epicoccum* were all more common outdoors as has been reported previously (Pasanen, 1992).

It is well recognised that much of the variation in indoor spore levels can be attributed to variation in outdoor levels (Burge, 1990). However, the present data suggests that while this is true in summer, outdoor levels are not a major influence on indoor spore levels throughout the year (Table 3). In fact, only 1% of indoor variation in viable spore levels was accounted for by outdoor levels in early spring (September), and only 3% of indoor total spore levels could be accounted for in late spring (November). It therefore becomes obvious that other factors have a significant influence on indoor spore levels, especially in cooler months, and it is likely that indoor sources of airborne spores is such an influence. Naturally, more frequent ventilation during summer combined with relatively higher outdoor spore levels would be part of the reason for a greater influence of outdoor spore levels in summer. However, the proportion of explained variance due to outdoor levels is not entirely seasonal, suggesting that the relationship is more complex. It is possible that environmental factors such as housing factors, humidity and temperature are significant influences on indoor spore levels, and such influences will be further evaluated.

In conclusion, the results from the present study show that airborne spore levels in homes in south-eastern Australia are relatively high throughout the year, but significant variation in levels and genera occur between seasons. Such variation is likely to be of significance for health implications caused by exposure to fungal spores in the home environment.

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