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A year-round comparison of fungal spores in indoor and outdoor air

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Abstract: This study was conducted by trapping airborne fungal spores inside and outside 15 residences in Kitchener-Waterloo, Ontario, Canada, monthly from December 1991 to September 1993. The dominant fungal propagules recorded indoors were *Cladosporium* (38.8%), *Aspergillus/Penicillium* (19.8%), *Leptosphaeria* (7.9%), unidentified basidiospores (6.5%), unidentified ascospores (2.8%), *Ganoderma* (2.6%), *Alternaria* (1.9%), *Coprinus* (1.8%), and *Epicoccum* (0.3%). Other unidentified spores (8.9%) and hyphal fragments (6.3%) also represented significant proportions of the total. Most common airborne spores were more numerous outside residences, except for those of *Aspergillus/Penicillium.*

Most fungal taxa recorded indoors showed seasonal periodicities similar to those in outdoor air, but with lower counts in summer than those recorded outdoors, except for *Aspergillus/Penicillium* and hyphal fragments. *Aspergillus/Penicillium* spores showed no seasonal patterns.

Key Words: airborne fungi, indoor, outdoor, seasonal periodicity

INTRODUCTION

The incidence of allergic respiratory disease is increasing in many countries, and airborne fungal spores are believed to be responsible for causing a significant proportion of such disease, although strict cause and effect relationships are difficult to prove. Most people in developed countries spend more than 90% of their time indoors, and in Canada, which has a long, cold winter, the significance of indoor air quality is obvious (Dekker et al., 1991). Despite this, most aeromycological studies have been conducted outdoors. If respiratory allergies are to be treated effectively, knowledge of airborne fungi indoors is perhaps even more important than that concerning propagules outdoors (Ebner et al., 1992). Few studies have focused on fungal spores both indoors and outdoors at the same time and in the same area (Ebner et al., 1992).

Airborne fungi indoors can originate both from outdoor and indoor sources. Lehtonen et al. (1993) showed that, from spring to fall, outdoor air is an important source of indoor fungal spores and, moreover, that concentrations of most spores indoors are mainly reflections of those outdoors. However, in winter, when the ground is covered by snow, indoor spore counts are higher than those outdoors (Pasanen et al., 1990; Reponen et al., 1992). The extent to which fungal spores in outdoor air infiltrate into buildings or residences is not fully understood (Ligocki et al., 1993), and the relationships between the indoor and outdoor aeromycota remain to be fully defined.

The objectives of this study were to determine quantitatively and qualitatively fungal spore numbers and seasonal periodicities within residences and to compare these data with that obtained in nearby outdoor environments.

MATERIALS AND METHODS

Fifteen residences in Kitchener-Waterloo, Ontario, Canada, were selected as indoor air-sampling sites. Twelve of the 15 residences housed patients with respiratory allergies and known sensitivity to mould spores. At each residence, on each sampling date, air samples were taken from six sites: living room, kitchen, bedroom, bathroom, family room and outdoor. Three residences were apartments and did not have family rooms, so only 5 sites were sampled. Samples were taken once a month at each residence between 1:00 PM and 9:30 PM. Three 10-min samples were taken with a Samplair-MK1 or -MK2 particle sampler (Allergenco, San Antonio, Texas) at each site. Samplers were placed on a table 50–80 cm in height for indoor sampling. For outdoor sampling the sampler was placed on the ground. Three samplers drawing 9.0, 15.0 and 15.5 L of air/min (factory calibration) were used in this study. Samplers were assigned to the rooms randomly at each sampling date to avoid sampler effect. The study was conducted from December 1991 to September 1993. In 10 residences the sampling continued for at least a year, but, either because the hous-

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TABLE I. Average airborne fungal spore abundances indoors and outdoors in the Kitchener-Waterloo area from 132 sampling days between Dec. 1991 and Sept. 1993

Taxa	Indoor		Outdoor	
	Spores/m³	%	Spores/m³	%
DOMINANT TAXA				
Alternaria	44	1.9	74	2.1
Aspergillus/Penicillium	457	19.8	131	3.8
Unidentified ascospores	65	2.8	138	4.0
Unidentified basidiospores	152	6.5	310	8.9
Cladosporium	895	38.8	1479	42.5
Coprinus	41	1.8	78	2.3
Epicoccum	7	0.3	20	0.6
Ganoderma	59	2.6	111	3.2
Hyphal fragments	146	6.3	112	3.2
Leptosphaeria	182	7.9	547	15.7
Other unidentified spores	206	8.9	301	8.7

es were sold or the patients withdrew from the study, the remainder were sampled for only 6 to 9 months. Residents were requested to perform only routine daily activities during the sampling periods. At each sampling site and date, temperature and relative humidity (RH) were recorded.

The slides used in sampling were coated with a thin layer of a mixture of 90% vaseline and 10% high melting point wax (w/w) and subsequently mounted with polyvinyl lactophenol under a coverslip. All fungal spores from the samples were counted and identified under the 40× and 100× objective of a Nikon light microscope with phase contrast optics. Data collected and analyzed included: 1) conidia of *Alternaria, Aspergillus/Penicillium, Cladosporium,* and *Epicoccum*; ascospores of *Leptosphaeria,* basidiospores of *Coprinus* and *Ganoderma*; 2) other ascospores and basidiospores which could not be identified to genus; and 3) other unidentified spores (neither ascospores nor basidiospores), hyphal fragments, total fungal spores and total number of genera. Since the conidia of *Aspergillus* and *Penicillium* cannot generally be distinguished from each other under a light microscope, these two genera were recorded as one pooled taxon. Airborne spores of recognizable taxa found only occasionally, of which there were 28, were recorded but not included in the analysis.

RESULTS

Temperature.—The mean annual temperature in residences in the Kitchener-Waterloo area was 21.1 ± 0.1 C, with extremes of 18.8 C and 23.7 C. The average temperature in summer was around 22 C and in winter around 19 C.

Relative humidity.—The mean relative humidity in the residences was 41.2 ± 0.4% over 1 year, with extremes of 33.1% and 55.2%. Relative humidity was highest, over 50% in summer, especially in July, August and September. The lowest RH values, around 35%, were recorded from January to March.

Spore numbers.—The dominant airborne spores indoors were of *Cladosporium* (overall mean: 895 spores/m³, 38.8% of the total), *Aspergillus/Penicillium* (457 spores/m³, 19.8%), *Leptosphaeria* (182 spores/m³, 7.9%), unidentified basidiospores (152 spores/m³, 6.5%), unidentified ascospores (65 spores/m³, 2.8%), *Ganoderma* (59 spores/m³, 2.6%), *Alternaria* (44 spores/m³, 1.9%), *Coprinus* (41 spores/m³, 1.8%), and *Epicoccum* (7 spores/m³, 0.3%) (TABLE I). Other unidentifiable spores (206 spores/m³, 8.9%) and hyphal fragments (146 pieces/m³, 6.3%) also made up a significant portion of the aeromycota. Propagules recorded only occasionally represented 28 taxa, but made up fewer than 3% of total propagules.

By comparison, the spore counts of most of the common airborne fungi outdoors were higher than those indoors. However, *Aspergillus/Penicillium* spores were more than twice as numerous indoors as outdoors (TABLE I). *Leptosphaeria* ascospores ranked second outdoors, but third indoors. Other taxa occurred in similar rank order both indoors and outdoors.

Seasonal periodicities.—Among common airborne hyphomycetes, *Cladosporium, Alternaria* and *Epicoccum* shared similar seasonal periodicities (FIG. 1), *Cladosporium* and *Alternaria* peaking in August and *Epicoccum* in October. Their periodicities indoors were similar to those outdoors but with lower counts and a September peak for *Epicoccum* (FIG. 1). *Aspergillus/Penicillium* had three peaks indoors, one in January,

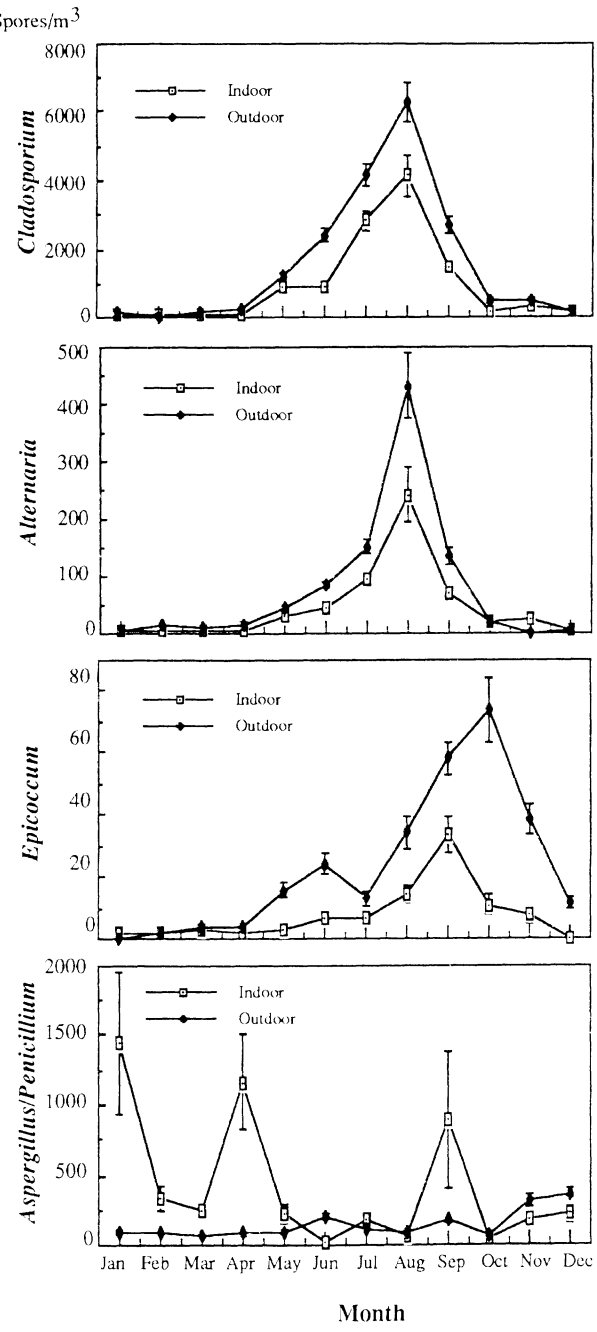

FIG. 1. Seasonal patterns of airborne conidia of *Cladosporium, Alternaria, Epicoccum* and *Aspergillus/Penicillium*. Error bars are mean ± SE.

a second in April and a third in September, but no perceptible peaks outdoors, remaining below 100 spores/m^3 most of the year (FIG. 1). This group was unusual in having generally higher counts indoors than outdoors.

Leptosphaeria ascospores were recorded only from July to September (FIG. 2). The outdoor counts were always higher than those indoors. The highest counts occurred in August (FIG. 2).

Unidentified airborne ascospores were recorded from March to November (FIG. 2) though counts decreased sharply in June, both indoors and outdoors. Again, indoor counts were always lower than outdoor counts, and the two increased and decreased concurrently.

Basidiospore counts of *Coprinus, Ganoderma* and unidentified taxa fluctuated similarly indoors and outdoors, with outdoor counts being higher (FIG. 2). Basidiospores of *Coprinus* were recorded from April to October; those of *Ganoderma* were up to three times more numerous and occurred from June to October (FIG. 2). Unidentified basidiospores were found almost year round, achieving very high numbers from July to October (FIG. 2). Basidiospore abundance outdoors fell sharply in August, a decrease reflected less dramatically indoors.

The seasonal periodicities of hyphal fragments found indoors and outdoors were somewhat divergent (FIG. 3). Indoor counts did not show a distinct seasonal pattern, though indoor and outdoor values peaked in August. Unlike most other airborne fungal propagules, counts of airborne hyphal fragments indoors were lower than those outdoors only from June to September; the rest of the time, hyphal fragments were more numerous indoors than outdoors. Only *Aspergillus/Penicillium* was comparable.

The abundances of unidentified spores displayed similar seasonal patterns both indoors and outdoors, with a peak period in August and September, and relatively high counts from April to October (FIG. 3). In winter, the spore concentrations indoors were slightly greater than those outdoors.

The seasonal patterns of total combined spores both indoors and outdoors were much the same, with a clear peak in August. From May to October the spore abundances outdoors were greater than those indoors. The rest of the time, spore counts were similar indoors and outdoors except in January, when the indoor count was higher (FIG. 3).

Biodiversity of identified airborne fungal spores both indoors and outdoors demonstrated seasonal patterns, with the highest numbers of taxa sporulating in summer and early fall (FIG. 3). In winter the numbers of common genera represented among airborne fungal spores were similar indoors and outdoors, and much lower than the totals from April to October. The common genera detected outdoors from April to October were more diverse than those indoors.

DISCUSSION

Abundances.—Significant differences were noted between abundances of airborne fungal propagules indoors and outdoors. It was apparent that not all indoor

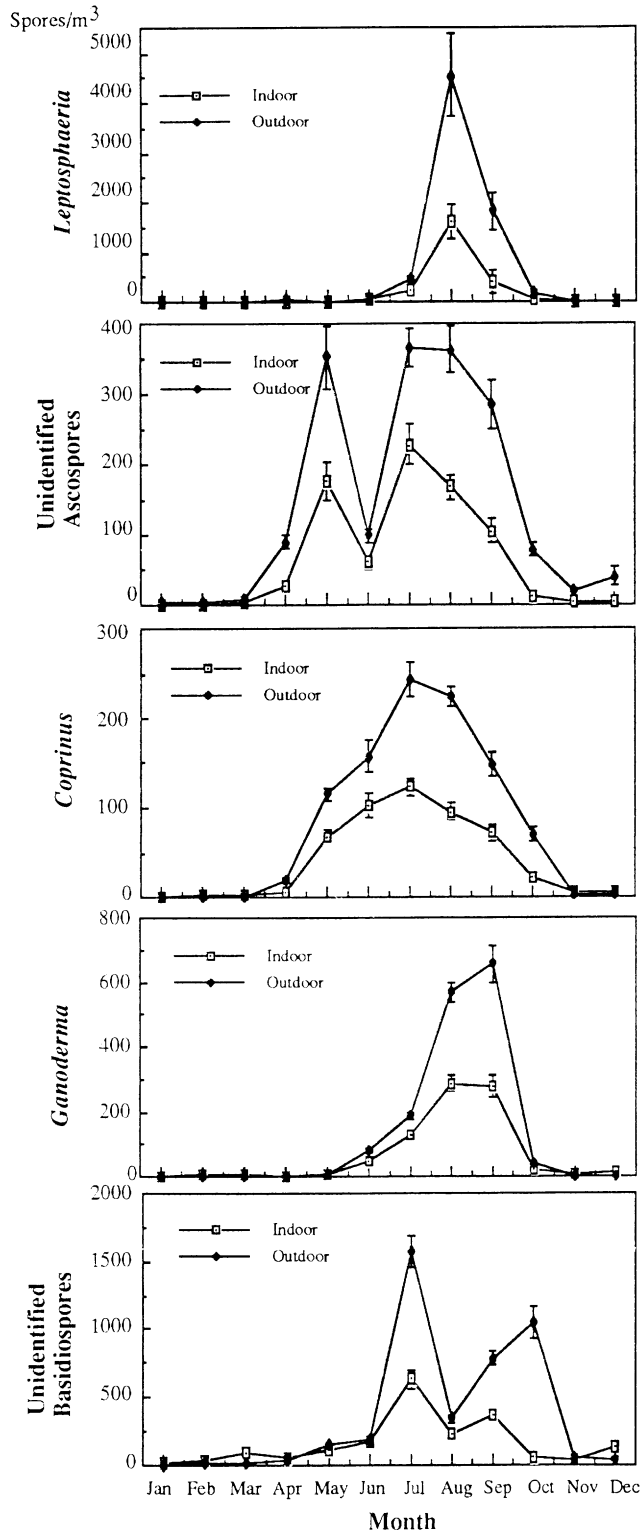

FIG. 2. Seasonal patterns of airborne ascospores of *Leptosphaeria,* unidentified ascospores, basidiospores of *Coprinus* and *Ganoderma,* and unidentified basidiospores. Error bars are mean ± SE.

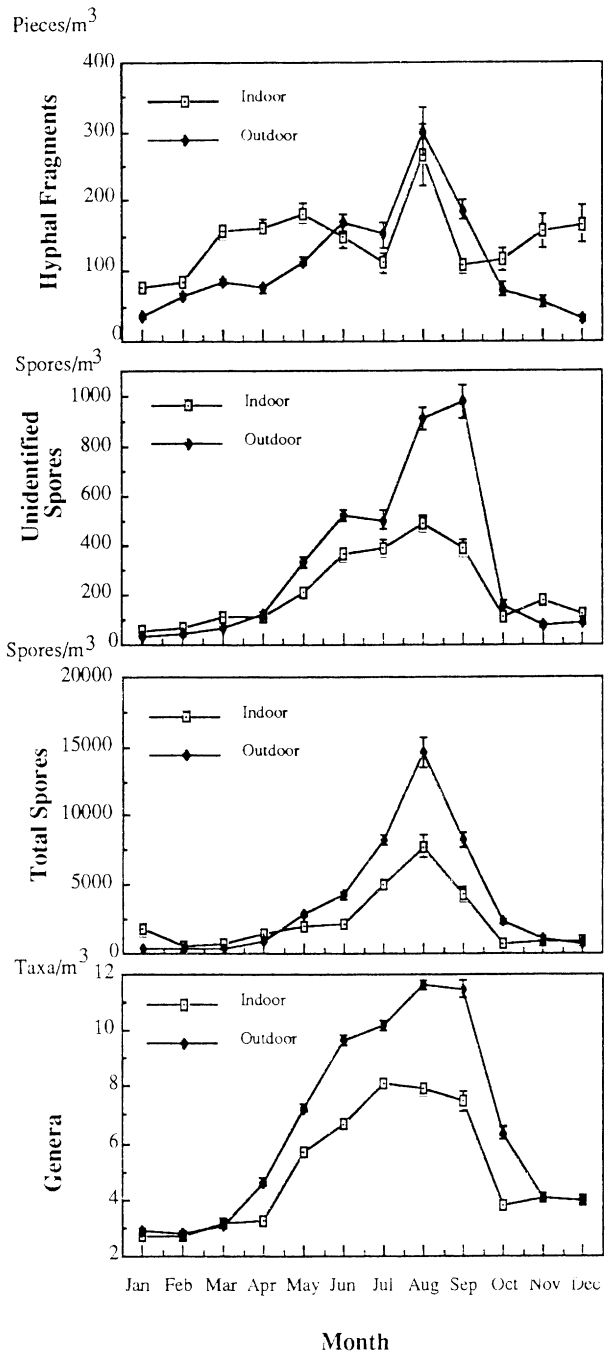

FIG. 3. Seasonal patterns of airborne hyphal fragments, unidentified spores, total spores and genera. Error bars are mean ± SE.

airborne fungi originated from outdoor sources, as is often assumed. A few taxa emerging from indoor sources, such as *Aspergillus/Penicillium*, were largely responsible for the differences. Nevertheless, the propagules of most taxa do appear to originate on outdoor substrates and are carried indoors with infiltrating air.

Seasonal periodicities.—Few observations of seasonal periodicities of airborne fungi in residences were found

in the literature. Nevertheless, in the present study, most of the fungal propagules encountered indoors, except for *Aspergillus/Penicillium,* displayed clearly defined seasonal periodicities similar to those observed outdoors, with peak periods in summer and low numbers in winter (FIGS. 1–3), which suggests a general relationship between airborne fungi indoors and outdoors. Outdoor spore sources are generally considered to make a major contribution to airborne populations indoors (Flannigan et al., 1991). In summer, spores can readily enter residences through open windows. In winter, however, residences in Canada are well insulated and sealed to conserve energy (Dekker et al., 1991). This reduced the opportunities for entry of airborne fungi. Fungal sources indoors are, therefore, very likely to be more important in winter than in summer.

In Austria and Spain *Cladosporium* spores showed different seasonal patterns indoors from those observed in the present studies (Ebner et al., 1992; Infante-Garcia-Pantaleton and Dominguez-Vilches, 1988). In Austria and Spain the seasonal patterns displayed a double peak with a minor trough around July and August. Differences in climate may account for this, since there was no rain in July and August in Spain (Infante-Garcia-Pantaleton and Dominguez-Vilches, 1988), but substrates may also be a factor.

The indoor seasonal pattern of *Alternaria* revealed in the present study was similar to that reported in Spain (Infante et al., 1987) except that the peak period in Spanish residences was around June, while in Kitchener-Waterloo, Canada, it was in August. The different weather patterns in the two countries probably account for this disparity.

The seasonal patterns of airborne fungal spores outdoors were mainly determined by their biological characteristics. *Epicoccum* and unidentified basidiospores peaked late and persisted until late fall (FIGS. 1, 2). The reason their spore counts indoors decreased much earlier than those outdoors was that temperature in October fell sufficiently that windows were closed, presumably excluding a large proportion of the spores. A similar indoor seasonal pattern for *Epicoccum* was found in Austria (Ebner et al., 1992).

Counts of *Aspergillus/Penicillium* conidia indoors did not correlate with those outdoors (FIG. 1). These conidia were usually more numerous indoors than those outdoors, suggesting that many of them were derived from indoor sources. Lehtonen et al. (1993) also noted that *Penicillium* occurred more commonly indoors than outdoors and thought that this was caused by indoor sources or by accumulation. A study using Andersen samplers in the Netherlands found that both *Aspergillus* and *Penicillium* had higher concentrations indoors than those outdoors (Beaumont et al., 1984), but another study using a jet spore sampler (viable method) in Austria found that the indoor spore concentration of *Penicillium* was usually lower than that outdoors, and that only in April and June were indoor spore counts of *Aspergillus* higher than those outdoors (Ebner et al., 1992). The seasonal patterns of *Penicillium* and *Aspergillus* indoors were different from those outdoors in Austria. Although both *Penicillium* and *Aspergillus* indoors showed multiple-peak seasonal patterns, the highest peaks of *Penicillium* were in summer, those of *Aspergillus*, in spring and early summer (Ebner et al., 1992). A study conducted in India found that *Aspergillus* peaked inside residences from June to November. Miller et al. (1988) found that *Penicillium* was the most common genus in both air and dust in Canadian houses from December to January. Since the sampling methods and sampling periods differed, it is difficult to make valid comparisons of those studies with the present one. Which species of *Aspergillus/Penicillium* are involved in homes, and which are more important to public health? Further studies are necessary to answer these questions.

Since species of *Leptosphaeria* are mainly plant pathogens (Hanlin, 1990), it is not surprising that its indoor and outdoor counts shared similar seasonal patterns. It is almost certain that airborne spores indoors were derived mainly from outdoor sources, unless house plants had become infected by *Leptosphaeria* sp. The spore concentrations of unidentified ascospores decreased dramatically in June. This may have been due to the fact that the rainfall in June, 1992 was 51 mm—far lower than the normal 77.7 mm. Burge (1986) noted that numbers of airborne ascospores increased with increased rainfall.

Ganoderma does not produce basidiomata indoors, and the seasonal patterns observed for *Ganoderma* basidiospores therefore indicate that spores trapped inside residences were from outdoor sources. Although *Coprinus domesticus* Fries has been found growing on an indoor substrate (van Bronswijk et al., 1986), this is not likely to be a common occurrence, and we may assume that spores of this genus recorded indoors originated from outdoor sources. The decrease in unidentified basidiospores in August cannot be explained satisfactorily from local meteorological data.

Earlier studies comparing airborne fungi inside and outside residences failed to find significant differences, so their authors suggested that the indoor spora was merely a reflection of that outdoors (Banerjee et al., 1987). This is probably true for most indoor fungi, but it does not apply to some, notably *Aspergillus* and *Penicillium.* But even when similar seasonal variations of indoor and outdoor spora are revealed, it cannot always be assumed that those indoors come entirely from outdoor sources.

Research on seasonal periodicities of indoor air spora will provide patients and doctors with information needed for controlling airborne fungal spore populations and will help to pinpoint the most important allergenic culprits in the indoor environment.

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LITERATURE CITED

Banerjee, U., P. Weber, J. Ruffin, and S. Banerjee. 1987. Airborne fungi survey of some residences in Durham, North Carolina, USA. *Grana* 26: 103–108.

Beaumont, F., H. F. Kauffman, H. J. Sluiter, and K. de Vries. 1984. Volumetric aerobiological study of seasonal fungus prevalence inside and outside dwellings of asthmatic patients living in the Northeast Netherlands. *Ann. Allergy* 53: 486–492.

Burge, H. A. 1986. Some comments on the aerobiology of fungus spores. *Grana* 25: 143–146.

Dekker, C., R. Dales, S. Bartlett, B. Brunekreef, and H. Zwanenburg. 1991. Child asthma and the indoor environment. *Chest* 100: 922–926.

Ebner, M. R., K. Haselwandter, and A. Frank. 1992. Indoor and outdoor incidence of airborne fungal allergens at low- and high-altitude alpine environments. *Mycol. Res.* 96: 117–124.

Flannigan, B., E. M. McCabe, and F. McGarry. 1991. Allergic and toxigenic micro-organisms in houses. *J. Appl. Bacteriol.* 70 (Suppl.): 61s–73s.

Hanlin, R. T. 1990. *Illustrated genera of Ascomycetes.* APS Press, St. Paul, Minnesota. 263 pp.

Infante, F., E. Dominguez, E. Ruiz de Clavijo, and C. Galán. 1987. Incidence of *Alternaria* Nees ex Fries in dwellings of Córdoba City (Spain). *Allergol. Immunopathol.* 15: 221–224.

Infante-Garcia-Pantaleton F., and E. Dominguez-Vilches. 1988. Annual variation of *Cladosporium* spores in home habitats in Córdoba, Spain. *Ann. Allergy* 60: 256–261.

Lehtonen, M., T. Reponen, and A. Nevalainen. 1993. Everyday activities and variation of fungal spore concentrations in indoor air. *Int. Biodeterior. Biodegrad.* 31: 25–39.

Ligocki, M., L. G. Salmon, T. Fall, M. Jones, W. W. Nazaroff, and G. R. Cass. 1993. Characteristics of airborne particles inside southern California museums. *Atmospheric Environm.* 27A: 697–711.

Miller, J. D., A. M. LaFlamme, Y. Sobel, P. Lafontaine, and R. Greenbalgh. 1988. Fungi and fungal products in Canadian homes. *Int. J. Biodeterior.* 74: 1115–1123.

Pasanen, A.-L., T. Reponen, P. Kalliokoski, and A. Nevalainen. 1990. Seasonal variation of fungal spore counts and genera in indoor and outdoor air in a subarctic climate. Pp. 39–44. *In: Indoor air '90.* Vol. 2. Ed., D. S. Walkinshaw. Canada Mortgage and Housing Corporation, Ottawa.

Reponen, T., A. Nevalainen, M. Jantunen, M. Pellikka, and P. Kalliokoski. 1992. Normal range criteria for indoor air bacteria and fungal spores in a subarctic climate. *Indoor Air* 2: 26–31.

van Bronswijk, J. E., G. Rijckaert, and B. van De Lustgraaf. 1986. Indoor fungi, distribution and allergenicity. *Acta Bot. Neerl.* 35: 329–345.