

Fungus Allergens Inside and Outside the Residences of Atopic and Control Children

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ABSTRACT. Airborne fungi were collected during the peak fungus season, using the N6 Andersen sampler inside and outside the homes of 46 asthmatic children, 20 atopic children, and 26 nonatopic control children in the Taipei area. The geometric mean fungus concentrations of the asthmatic, atopic, and control groups were found to be 565, 411, and 608 colony-forming units (CFU) per m³ in the living rooms; 659, 464, and 602 CFU m³ in the bedrooms; and 547, 449, and 668 CFU m³ outdoors; respectively. *Aspergillus*, *Cladosporium*, and *Penicillium* were observed to be the most common microfungi, both indoors and outdoors. High correlations between indoor fungus levels (living rooms or bedrooms) and the corresponding outdoor levels were observed for these predominant genera. With regard to the *Aspergillus* species composition, *A. flavus* and *A. niger* were observed to be predominant and are, therefore, recommended to be included on a skin test panel. Moreover, among these three groups, there were statistically significant concentration differences for *Cladosporium* and *Penicillium*.

ASTHMA AND RHINITIS are common chronic respiratory diseases that are often caused by allergen exposure, although organic dust or volatile chemicals can also induce allergic airway diseases.¹ It was found that the prevalence of asthma in Taipei schoolchildren increased from 1.3% in 1974 to 5.1% in 1985² and to 5.8% in 1992 (unpublished data). In addition, more than 45% of the asthmatic children and 70% of asthmatic adults were found to have positive allergic responses to molds (*Aspergillus fumigatus*, *Cladosporium*, *Penicillium*,

um, and *Alternaria*). Therefore, fungi are regarded as one of the predominant sources of allergens in Taiwan.

Our previous investigation³ demonstrated that seasonal variations of airborne fungus concentrations were remarkable, and indoor/outdoor air spore counts varied considerably from residence to residence in Taipei. Fungus concentrations in the summer were observed to be 20-25 times higher than corresponding levels during the winter. A large variety of mold genera were isolated, and *Aspergillus*, *Cladosporium*, *Penicillium*, and yeast

were observed to be predominant. Indoors, *Penicillium* showed the highest concentrations in the summer and autumn months, whereas *Aspergillus* and *Cladosporium* were also observed frequently. Outside air was dominated by *Aspergillus*, *Cladosporium*, and *Penicillium* in spring, summer, and autumn, but was dominated by *Penicillium* and yeast during the winter months. In addition, no *Cladosporium* was found indoors or outdoors in the winter. No data, however, have been reported concerning fungus characteristics inside and outside of atopic and nonatopic children's homes in Taiwan, which location has a subtropical climate. This report evaluated characteristics of fungus allergens inside and outside the residences of 46 asthmatic, 20 nonasthmatic atopic, and 26 nonatopic control children during the peak fungus season in the Taipei area. Fungus allergen concentration distributions and differences in fungus characteristics between atopic and nonatopic groups were compared.

Materials and Method

Subjects. Forty-six children, aged 7 to 15 y, who fulfilled the American Thoracic Society criteria for reversible airway disease⁴ (bronchial asthma) were recruited from the Pediatric Allergy/Immunology Division of the National Taiwan University Hospital. All of the children had at least one positive reaction to a panel of common inhaled aeroallergens: house dust mite, cotton, rice straw, feather, dog, cat, *C. albicans*, *A. fumigatus*, *Cladosporium*, *Penicillium*, *Alternaria*, ragweed, and cockroach. The other 20 nonasthmatic atopic patients (allergic rhinitis or atopic eczema) who

attended the allergic clinic of National Taiwan University Hospital were also included in the study. Informed consent was obtained from each asthmatic atopic patient, and the study was approved by the Human Research Committee of this hospital. Twenty-six primary school children, aged 7 to 12 y, who had no history of atopic diseases and who lived in the Taipei urban area served as the control group.

Air sampling and fungus identification. Houses of the 46 asthmatic, 20 atopic, and 26 nonatopic control children were visited from July, 1993, to mid-September, 1993. All of the homes investigated were normal multistory buildings and were occupied by middle-income families. During the air sampling, there were 2 to 3 occupants present, pets were not present, and there were no excessive human activities. In addition, no air conditioners were operated during the field measurements.

Andersen one-stage culture plate impactors (N6, Andersen Samplers, Inc.; Atlanta, GA) were used for indoor and outdoor measurements. The indoor and outdoor sampling locations were the living rooms/bedrooms and the balcony, respectively, of every residence; sequential duplicate measurements were conducted. The sampling height was 1.2 to 1.5 m above the floor and simulated the human breathing zone. During the air sampling, temperature and relative humidity were recorded. Sampling plates were prepared by pouring 20 ml of malt extract agar (MEA), aseptically into 100-mm diameter × 15-mm sterile plastic petri dishes, so that the gap between the nozzles and agar surface met the manufacturer's specification. We used a sampling time that ranged from 30 sec to 3 min, at a flow rate of 28.3

Table 1. Fungus Characteristics in the Homes of Asthmatic, Atopic, and Control Children

Genus/species	No. Homes with I/O ratio*			Geometric mean concentrations (CFU/m ³)								
				Asthmatic (n = 46)			Atopic (n = 20)			Control (n = 26)		
				Sampling locations								
	<1	1–2	>2	LR	BR	OUT	LR	BR	OUT	LR	BR	OUT
<i>A. flavus</i>	24	15	6	8.8	7.5	4.7	6.6	4.2	2.7	5.7	10.8	5.9
<i>A. niger</i>	33	12	3	4.7	5.3	4.7	2.8	5.3	6.7	3.9	3.0	5.9
<i>A. fumigatus</i>	3	0	0	1.2	—	1.1	1.1	—	1.1	1.3	1.1	1.2
<i>A. clavatus</i>	4	0	0	1.1	1.2	1.1	—	—	1.1	1.2	1.3	1.2
<i>Aspergillus</i>	37	26	12	24.1	24.6	17.2	15.8	21.7	22.5	15.8	21.5	23.6
<i>Penicillium</i>	41	29	17	72.9	62.1	47.6	35.8	45.7	55.5	94.1	78.6	101
<i>Cladosporium</i>	30	14	2	10.9	15.7	19.5	6.9	10.2	7.3	3.1	4.6	3.8
<i>Alternaria</i>	15	2	0	1.4	1.3	1.7	1.1	1.1	1.5	1.2	1.2	1.8
<i>Paecilomyces</i>	20	3	1	1.7	2.4	2.2	1.9	1.9	1.5	1.5	2.1	2.0
<i>Curvularia</i>	19	0	0	1.5	1.4	1.7	1.5	1.8	1.3	—	1.3	1.9
<i>Fusarium</i>	34	7	2	3.3	4.2	4.3	3.1	2.6	1.8	2.0	2.5	5.5
<i>Trichoderma</i>	4	1	0	—	—	1.1	—	—	1.1	1.1	1.1	1.2
Yeast	35	26	20	104	124	70.3	76.5	92.6	73.8	83.6	108	72.5
Total	51	28	10	565	659	547	411	464	449	608	602	668

Notes: LR = living room, BR = bedroom, and OUT = outdoors.

*I/O ratio = living room/outdoor ratio; the sample with outdoor concentration of zero is not included.

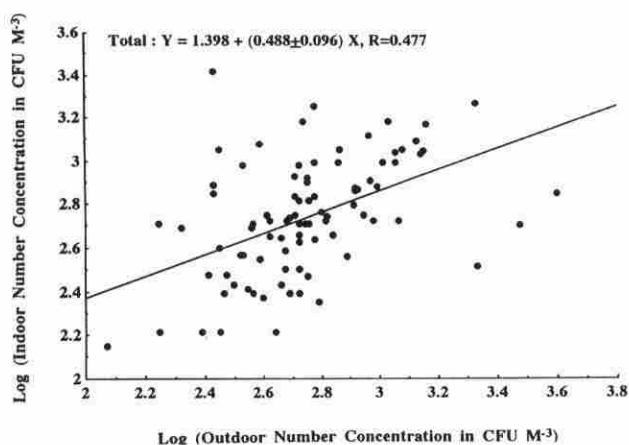


Fig. 1. Relationships between culturable airborne fungi found in the outdoors and living rooms (indoors).

l/min, to obtain the appropriate surface density for counting and identification.⁵ MEA is used widely for fungus collection and is recommended by the ACGIH committee for indoor environments.⁶ MEA contains 20 g/l malt extract, 1 g/l peptone, 20 g/l dextrose, and 20 g/l agar and has a final pH of 4.5–5.0. After the field measurements, the plates were incubated at a temperature of 25 °C for 4–6 d. The fungus counts (including yeast) are presented as colony-forming units in air (CFU/m³). Values of CFU/m³ were obtained, using the positive hole conversion table⁷ and by calculating the volume of air sampled. Form *Aspergillus* species and additional taxa were identified from slide cultures with standard references.⁸

Results

The airborne fungus allergens were evaluated inside (living rooms and bedrooms) and outside of the houses of 46 asthmatic, 20 atopic, and 26 nonatopic control children during the summertime (peak fungus season). During the field sampling period, the air temperature was in the range of 24–34 °C, and the relative humidity ranged from 56% to 90%. The concentrations of each of the fungus allergens identified varied widely. The concentration distributions were right-skewed and became distributed normally after ln-transformation. The geometric mean (GM) and indoor/outdoor (I/O) ratio ranges of the fungus concentrations in atopic and control groups are shown in Table 1. The GM fungus concentrations of the asthmatic, atopic, and control groups were found to be 565, 411, and 608 CFU/m³ in the living rooms; 659, 464, and 602 CFU/m³ in the bedrooms; and 547, 449, and 668 CFU/m³ outdoors; respectively. On the other hand, the I/O ratio is an indicator for evaluating the difference between indoor and outdoor fungus characteristics. Most of the identified genera had few samples with an I/O ratio larger than 2, excluding *Aspergillus*, *Penicillium*, and yeast.

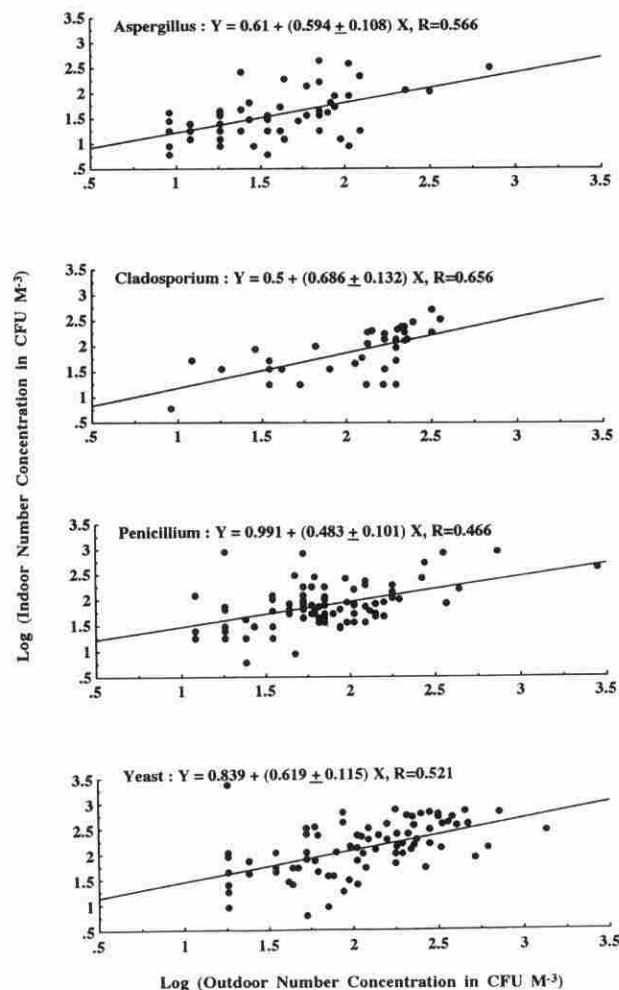


Fig. 2. Relationships between *Aspergillus*, *Cladosporium*, *Penicillium*, and yeast found outdoors and in living rooms (indoors).

The relationship between indoor and outdoor paired fungus concentrations for total collected samples ($n = 92$) was tested, using a paired t test (Fig. 1). The Pearson correlation coefficients (r_s) were .477 ($p = .0001$) and .400 ($p = .0002$) between outdoors and living rooms and outdoors and bedrooms, respectively. In addition, r_s of culturable airborne fungi between outdoors and living rooms were observed to be .566 ($p = .0001$) for *Aspergillus*, .656 ($p = .0001$) for *Cladosporium*, .466 ($p = .0001$) for *Penicillium*, and .521 ($p = .0001$) for yeast (Fig. 2). Moreover, the correlations between bedrooms and outdoors for *Aspergillus*, *Cladosporium*, *Penicillium*, and yeast were found to be .574 ($p = .0001$), .614 ($p = .0001$), .392 ($p = .0002$), and .557 ($p = .0001$), respectively.

The differences in fungus characteristics between the homes of atopic and control children were evaluated. It was found that the median fungus concentrations of asthmatic, atopic, and control groups were found to be 525, 468, and 700 CFU/m³ in the living rooms; 653, 426, and 525 CFU/m³ in the bedrooms; and 525, 490, and 562 CFU/m³ outdoors; respectively (Fig. 3). Moreover, the concentrations of asthmatic and control

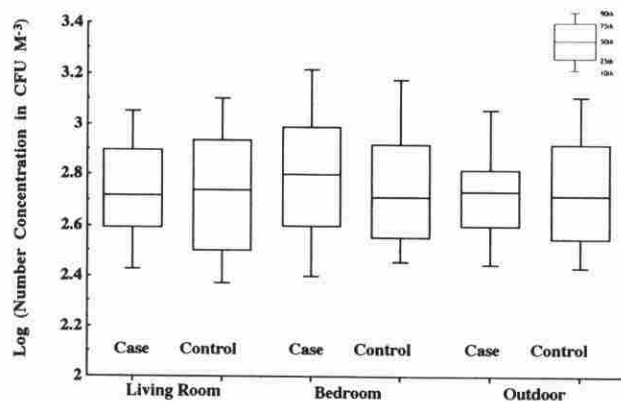


Fig. 3. Concentration distributions of total fungi in the homes of asthmatic, atopic, and control children; (* $p < .05$).

groups in living rooms and bedrooms were observed to be statistically significantly higher than those in atopic groups. The concentration distributions of the isolated predominant genus, *Aspergillus*, *Cladosporium*, *Penicillium*, and yeast are shown in Figure 4. The indoor and outdoor median concentrations of *Aspergillus*, *Cladosporium*, *Penicillium*, and yeast were in the range of 25–48 CFU/m³, 38–168 CFU/m³, 42–102 CFU/m³, and 97–230 CFU/m³, respectively. It was found that statistically significant concentration differences for *Cladosporium* and *Penicillium* occurred among these three groups. In addition, the predominant *Aspergillus* species were observed to be *A. flavus* and *A. niger*, with a median concentration of 18 CFU/m³ (Fig. 5).

Discussion

In this field investigation, the indoor and outdoor fungus characteristics were evaluated in a subtropical area, and the fungus allergen differences in the homes between atopic and nonatopic children were also assessed. Indoor fungus concentrations observed in this study were comparable with those observed in normal buildings or homes in Alabama and Tennessee,⁹ Edinburgh,¹⁰ Finland,¹¹ the Netherlands,^{12,13} midwestern United States,¹⁴ San Francisco,¹⁵ and Toronto.¹⁶ With regard to the outdoor fungus concentrations, the levels were lower than those of normal buildings or homes in Alabama and Tennessee,⁹ the Central California coast,¹⁷ Finland,¹¹ Italy,¹⁸ and Toronto.¹⁶ Moreover, there was no significant relationship between the fungus concentrations measured and relative humidity. This observation might be related to the high relative humidity in Taiwan.

With regard to fungus genus, *Penicillium* was the most prevalent isolated genus. Yeast were the second most frequent, occurring in more than 90% of both the indoor and outdoor samples. *Aspergillus* was next in frequency, occurring in 80% of the total collected samples. Furthermore, the occurrence percentages for *Cladosporium*, *Fusarium*, *Paecilomyces*, *Curvularia*, and *Alternaria* were observed to be 50%, 42%, 26%, 12%, and 15%, respectively. It was observed that *Aspergillus*

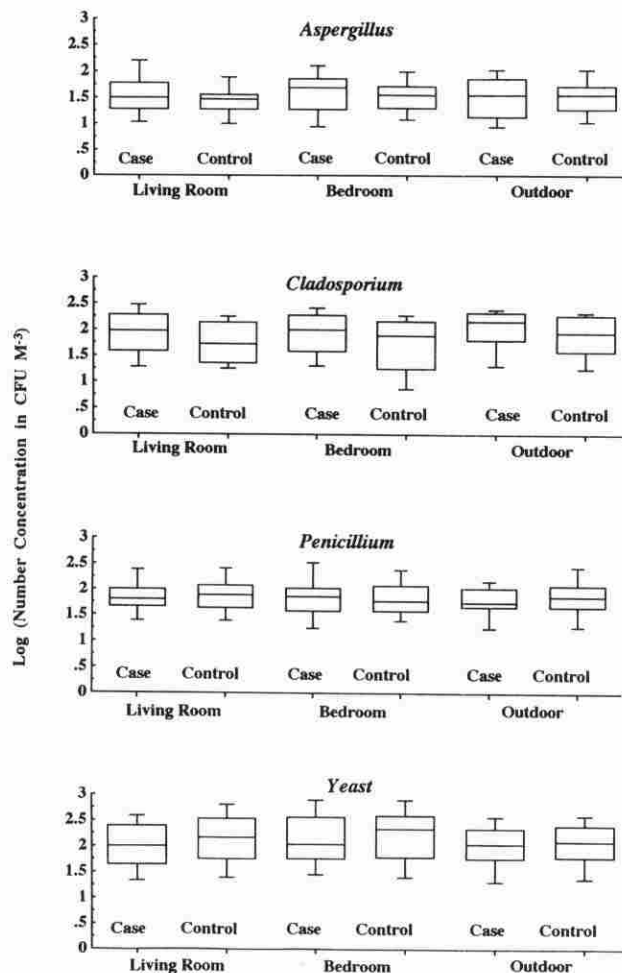


Fig. 4. Concentration distributions of *Aspergillus*, *Cladosporium*, *Penicillium*, and yeast in the homes of asthmatic, atopic, and control children; (* $p < .01$).

levels were comparable with those observed in Toronto¹⁶ and the Netherlands.¹² The concentrations of *Penicillium* in Taipei were lower than the results reported by the Netherlands¹² and were comparable with the levels in Toronto.¹⁶ Moreover, the *Cladosporium* levels observed were lower than those measured in Italy,¹⁸ the Netherlands,¹² and Toronto.¹⁶

Aspergillus species associated with decaying vegetable matter are considered to be human pathogens related to hypersensitivity pneumonitis¹⁹ and extrinsic allergic alveolitis.²⁰ *A. fumigatus* spores especially have been observed to be involved in a number of clinically important diseases.²¹ In addition, *A. flavus* and *A. parasiticus* have been reported to contain a high amount of aflatoxin.²² In this study, *A. flavus* and *A. niger* were found to be predominant, with more than a 50% occurrence frequency, but the occurrence rates of *A. fumigatus* and *A. clavus* were only 4%. The GM concentrations for these mainly isolated species, *A. flavus*, *A. niger*, *A. fumigatus*, and *A. clavus*, were 20 CFU/m³ (13–38 CFU/m³), 20 CFU/m³ (9–40 CFU/m³), 9 CFU/m³ (5–30 CFU/m³), and 12 CFU/m³ (8–30 CFU/m³),

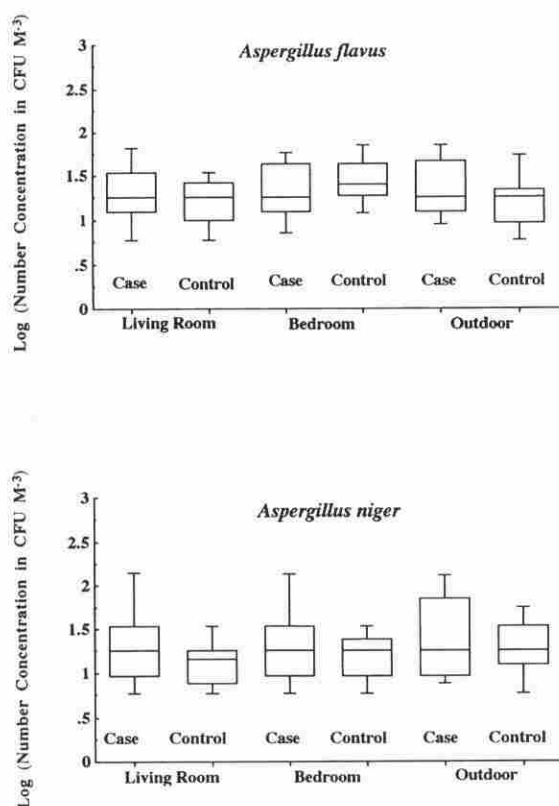


Fig. 5. Concentration distributions of *A. flavus* and *A. niger* in all of the homes in which measurements were conducted.

respectively. Moreover, *A. fumigatus* and *A. niger* levels were found to be higher than those measured inside and outside of the university hospital in Michigan.²³ From the skin tests to a variety of mold in 1 000 asthmatic children in Taiwan,²⁴ the positive rates of *A. fumigatus*, *Cladosporium*, *Penicillium*, and *Alternaria* were observed to be 26.7%, 22.8%, 22.3%, and 16.7%, respectively. Although the levels of *A. fumigatus* were found to be relatively low in Taipei, the positive rates were still quite high. This might be related to the fact that this particular fungus has outstanding allergen properties.²⁵ The results in this investigation suggest that the observed predominant *Aspergillus* species, *A. flavus* and *A. niger*, might be included in future skin test panels to insure more accurate evaluation of the positive rates of asthmatic patients to mold allergens.

Few data have been available regarding the comparisons of atopic and nonatopic children with fungus exposure. In Stockholm,²⁶ considerably less dust-bound fungi were observed in the homes of atopic children than in homes of control children. Their observation was explained by intense house cleaning by the parents of the atopic children. In our investigation, there were statistical concentration differences for *Cladosporium* and *Penicillium* in the houses of atopic patients, compared with those measured in the homes of the healthy individuals. Further investigation is needed to evaluate the relationships between health symptoms and viable microfungi characteristics in Taiwan.

In summary, our field study of airborne fungi indoors and outdoors indicated that a high amount of fungus allergens is present in the houses of the Taipei urban area. *Aspergillus*, *Cladosporium*, *Penicillium*, and yeast were isolated mainly from the airborne viable samples in Taiwan. Moreover, there were statistically significant differences in the concentration distributions of *Cladosporium* and *Penicillium* between the atopic and control groups. Therefore, procedures to reduce fungus allergens to as low a level as possible might be a feasible way to prevent the continuing rise in the prevalence of allergy in this area. In addition, further investigation of the relationships between fungus exposure and the occurrence of allergic symptoms is needed.

* * * * *

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