

Personal exposure to allergenic pollen and mould spores in inland New South Wales, Australia

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Summary

Background In inland NSW, Australia, allergic sensitization to the fungi *Alternaria* and *Cladosporium* and to pollen is common and an important risk factor for asthma.

Objective We report the results of a series of experiments designed to assess the nature of personal exposure to these airborne allergenic particles. We have tested the effect of exposure conditions and level of activity on measurements of the personal exposure.

Method Personal Air Samplers (PAS) and Nasal Air Samplers (NAS) were employed. NAS are fitted just inside the nose and collect inhaled particles by impaction, while the PAS use a pump-operated filter with constant air flow (2 L/min). Thirty-three subjects (adults and children) used both NAS and PAS simultaneously for four one hour periods during which they performed activities or rested, both inside and outside their homes. Samples were analysed by light microscopy. *Alternaria* spores, *Cladosporium* spores, grass pollen and nongrass pollen were counted.

Results Both samplers detected substantial variation in exposure between subjects. Between members of the same household, the intrahouse correlation coefficient ranged from <0–0.38. Levels of pollen grains and fungal spores inhaled were higher during periods of activity than during rest, and higher while subjects were outdoors than indoors. During the active outdoor period, the number of *Alternaria* spores inhaled ranged from 4 to 794 (median 11) spores/hr, *Cladosporium* from 0 to 396 (median 4) spores/hr, grass pollen from 0 to 81 (median 1) grains/hr and nongrass pollen from 0 to 72 (median 5) grains/hr.

Conclusion This is the first study to quantify individual inhaled levels of allergenic fungal spores and pollen under normal domestic circumstances. Exposure can be substantial and highly variable between individuals. The amount of particles inhaled relates both to location of the individual and activity being performed, independent of age group.

Keywords: *Alternaria*, *Cladosporium*, pollen, grass pollen, nasal air sampler, personal air sampling, aeroallergen, Burkard trap, IOM

Clinical and Experimental Allergy, Vol. 30, pp. 1733–1739. Submitted 20 August 1999; revised 11 April 2000; accepted 19 May 2000.

Introduction

Much of our understanding of the nature of human exposure to pollen grains and fungal spores has been derived from measurements of their airborne concentrations performed at a single site within a community. Such measurements are considered to provide a proxy measurement for all people

within that community. Indeed use of this information is increasing with daily pollen and fungal counts now available by postal zones from internet sites and increasing attention has been paid to the spacial distribution of such monitoring stations in order to provide a representation of regional exposures.

However there is little data on the fine detail of exposure to such aerospora, particularly on the differences which occur within population groups. Not only are the magnitude of differences occurring within the community unknown,

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but the causes of these differences are not identified. Thus little is known about the impact of subject age, location or activities on exposure. To study this personal exposure would require simultaneous measurements conducted within the breathing zone of numerous individuals. In the few studies of personal allergen exposure which have been performed, usually on individuals in the course of occupational health studies, it has shown that marked differences in exposure do occur over short distances and that sampling at a static location is only weakly representative of individuals' actual personal exposure [1,2].

We were interested in personal exposure to the major aeroallergens *Alternaria*, *Cladosporium* and pollen in a rural community in inland Australia. Here allergy to both grass pollen and fungi were common [3], and sensitization to the fungus *Alternaria* had been identified as an important risk factor for asthma in this community [4].

Previously it has been difficult to explore personal exposure because of practical limitations of the sampling technology. We have recently developed a simple method for sampling exposure which consists of small filters which fit just inside each nostril [5]. These collect inhaled particles by impacting them onto an adhesive film. The devices have been previously used to capture inhaled particles carrying cockroach [6], house dust mite [7] and cat allergen [8]. We used these devices, together with standard personal samplers, to investigate variation in personal exposure to a range of pollen and fungi among families participating in a set routine of activities both indoors and outdoors.

Materials and methods

Subjects and setting

The study was conducted in Moree, New South Wales, Australia over four warm, dry days in November, 1997, during peak spore and pollen seasons. Moree is an inland town in which dry (average summer RH 20%) and hot conditions (average daily summer maximum temperature 35 °C) are associated with high ambient daily concentrations of *Alternaria* spores. The study was approved by the Human Research Ethics Committee of the University of Sydney.

The study was conducted in nine family groups each comprising two adults and two children who could all comfortably wear the Nasal Air Samplers (NAS) (nostril orifice ~ 5 × 10 mm). The mean age of adults was 36 years and the mean age of children was 10 years. The youngest child was an 8-year-old. During the experiments four children proved unable to wear the NAS and in two of these cases the researcher conducting the study substituted for the child. All 36 subjects also wore a Personal Air Sampler (PAS) which consisted of a portable pump and filter. Three of the four

children who did not wear NAS did wear the PAS. Overall, in the nine family groups of four, 20 adults and 14 children wore NAS and 18 adults and 17 children wore PAS.

Air sampling

Nasal air samplers The NAS consisted of small, moulded devices that seal into each nostril and collect inhaled particles by impacting them onto an internal collection plate ('backing plate') which were covered by a clear, sticky film of silicone grease (Dow Corning, Molycote 111). The samplers have low air resistance with highest efficiency obtained collecting particles over 8 µm in aerodynamic diameter [5]. The devices are manufactured by the Institute of Respiratory Medicine (Australia).

Personal air samplers The PAS were conventional systems widely used in occupational medicine. They consisted of a small battery-operated pump (SKC Limited, model PCXR4) drawing a constant flow of air at 2.0 L per minute through a 0.8-µm membrane filter (Millipore, Lane Cove, NSW) held within an IOM sampling head (SKC Limited, Dorset, UK) [9]. The sampling head was worn on subject's right lapel within the breathing zone, while the pump was worn on the belt. The samplers were used in accordance with the occupational standards AS2985-1987 and AS3640-1989.

Burkard volumetric trap sampling

Ambient exposure to fungal and pollen particles during the experimental period was measured using a Burkard spore trap positioned in the town centre, 12 m above ground level on the roof of the Moree Community Hospital and operated in accordance with standard AS 2922-1987. Particles impacted onto melinex tape coated with silicone grease (Dow Corning, Molycote 111).

Experimental design

Both NAS and PAS were simultaneously used by each subject over four sets of 1 hour sampling periods. Two samples were collected inside the house and two outside the house. At both locations, the subjects engaged in either a low level activity or a moderate level activity, each for an hour. Indoor low level activities included reading, dozing, watching television and playing board games while low level outdoor activities usually involved sitting and reading. Moderate level activities indoors involved cooking, cleaning and walking around the house whereas the hour of moderate level activity outdoors included gardening and walking. None of the activities resulted in exercise at an intensity where mouth breathing occurred. All activities

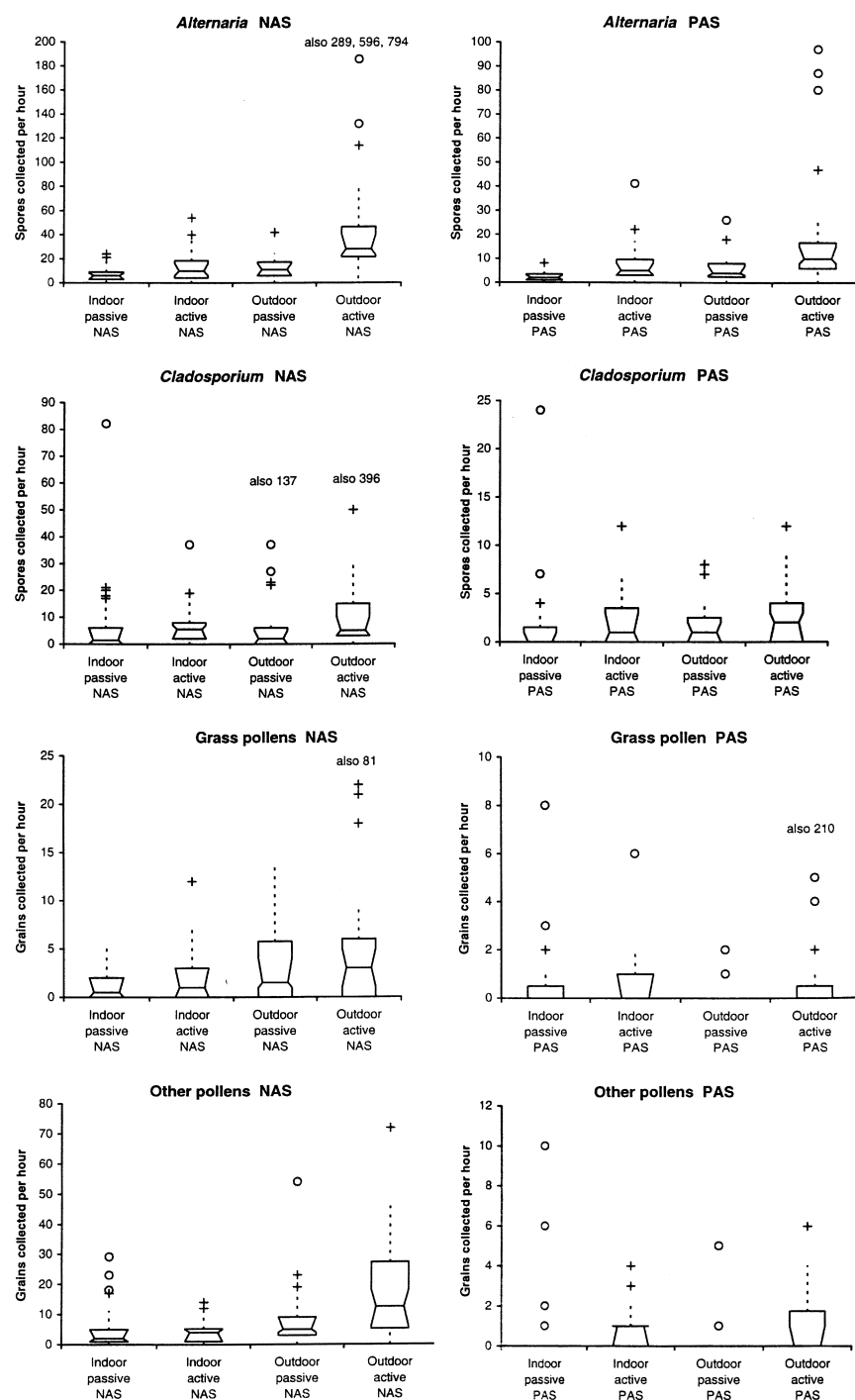


Fig. 1. Distribution of individual spore and pollen counts sampled per hour using NAS and PAS, grouped by activity and location. Key to box-plot outlier symbols: (— — —) connects observations within 1.5 interquartile ranges (IQRs) of both upper and lower quartiles; (+) near outliers, between 1.5 and 3.0 IQRs away; (o) far outliers, greater than 3.0 IQRs away note: indents extending from median lines indicate confidence intervals.

were chosen by the subject as being typical of how they spent their time.

Sample analyses

Staining and counting Unstained preparations of all nasal and personal air samples were assessed by light microscopy. Entire filters and backing plates were viewed at 250×

magnification. The four categories of particles counted were *Alternaria* spores, *Cladosporium* spores, grass pollen grains and nongrass pollen grains. For NAS counts, each subject's left and right nostril exposures were summed, except in three cases where one member of a sample pair was damaged. In these cases the undamaged count was doubled. (This assumption is reasonable as repeatability of left and right NAS samples, expressed as intraclass correlation

coefficient (see below), was 0.92 for *Alternaria* spore counts and 0.76 for total particles).

Statistical analyses

Data were analysed using the Statistical Analysis System (SAS) software package (SAS Institute Inc., Cary, NC, USA) and plotted using Analyse-It for Microsoft Excel (Analyse-It Software Ltd, Leeds, UK). As the distribution of particle counts was skewed to the right and included zero values, the counts were square root-transformed for analysis of variance. Means and standard deviations were calculated on square-root transformed counts and reported on the back-transformed values. Variation among members of the same household, that is within-house variation, was assessed using the intraclass correlation coefficient (ICC), derived from analysis of variance [10].

The correlation between the NAS and the PAS was calculated using Spearman partial correlation coefficient, adjusting for level of activity (passive, active) and location (indoor, outdoor). The relation between the two methods (agreement) was quantified using the method of Bland and Altman [11]. As the data were skewed, $\log_{10}$ transformation was applied to the absolute counts plus 1. The average difference in log counts was calculated after adjustment for level of activity and location. The effects of age group (child and adult), location and level of activity on NAS and PAS particle counts were tested by an analysis of variance for repeated measures implemented using PROC MIXED in SAS with a term for family as well as for each of the main effects of interest.

Possible interactions between age group, location and level of activity were also tested.

Results

The ambient counts for mould spores and pollen grains in the community during the study period were moderate. The daily Burkard volumetric trap counts ranged from 59 to 197/m³ for *Alternaria* spores, 542–1457 spores/m³ for *Cladosporium*, 24–48 grains/m³ for grass pollen and 70–383 grains/m³ for nongrass pollen over this 4-day period.

Detection of particles

The numbers of the four types of particles collected on the NAS by inhalation over one hour are shown on the left hand side of Fig. 1, grouped by activity level and location. The numbers of *Alternaria* spores inhaled in this period ranged between 0 and 794 (median 11) spores, *Cladosporium* 0–396 (median 4) spores, grass pollen 0–81 (median 1) grains and nongrass pollen 0–72 (median 5) grains.

Table 1. Within family correlation (intraclass correlation coefficient) of the nasal air sampler and personal air sampler data. Total particles is the sum of the counts of *Alternaria* spores, *Cladosporium* spores, grass and nongrass pollen

Activity	<i>Alternaria</i> spores/hr		Total particles/hr	
	NAS	PAS	NAS	PAS
Indoor passive	0.37	0.08	0.25	0.06
Indoor active	0.04	0.17	–0.04	0.09
Outdoor passive	0.04	0.38	–0.03	0.47
Outdoor active	0.15	–0.06	–0.003	–0.07

The corresponding collection of the four types of particles collected by the PAS are shown down the right hand side of Fig. 1. The numbers of *Alternaria* spores sampled ranged between 0 and 97 (median 4) spores, *Cladosporium* 0–24 (median 1) spores, grass pollen 0–210 (median 0) grains and nongrass pollen 0–10 (median 0) grains.

Other fungi collected by both NAS and PAS were *Curvularia*, *Torula*, *Exserohilum*, *Pithomyces*, *Bipolaris*, *Spegazzinia*, *Pleospora*, *Epicoccum*, *Beltrania* and a few unidentified ascomycetes.

The extent to which exposure measurements on one individual in a house is representative of the exposure of all members of that household was assessed by examining the correlation among members of the same household breathing in the same environment (Table 1). Overall intra-house correlation coefficients were low for both the NAS and the PAS, indicating that individual factors influenced subjects' exposure, even within the same local environment.

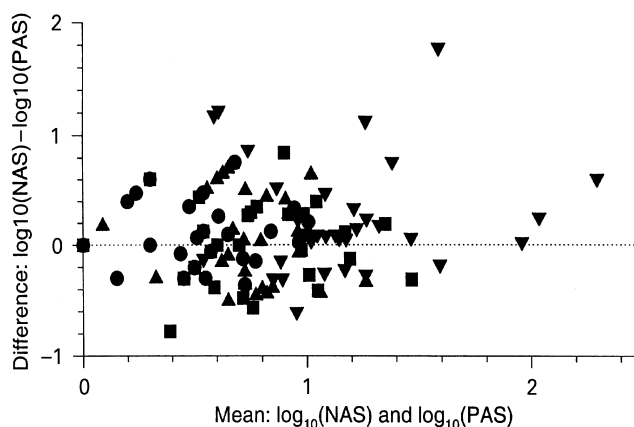


Fig. 2. Distribution of the difference between NAS and PAS *Alternaria* spore counts sampled per hour, against the mean of the NAS and PAS spore counts sampled per hour. (●) Indoor passive; (■) indoor active; (▲) outdoor passive; (▼) outdoor active.

Table 2. Difference between groups and conditions of particle counts by NAS (by ANOVA)

NAS	Mean* <i>Alternaria</i> spores/hr (95% CI)	P-value	Mean* total particles/hr (95% CI)	P-value
Children	14.5 (11.3,18.1)	0.63	30.6 (24.3,37.6)	0.40
Adults	13.1 (9.6,17.2)		26.1 (19.2,34.0)	
Indoors	8.6 (6.6,10.9)	0.0001	18.0 (13.8,22.8)	0.0001
Outdoors	20.3 (15.7,25.6)		41.7 (32.9,51.6)	
Passive	8.5 (6.8,10.3)	0.0001	21.3 (16.4,27.0)	0.001
Active	20.5 (15.5,26.2)		36.1 (27.7,45.7)	

*Means were calculated on square root transformed values and the results were back-transformed to the original scale of measurement.

Table 3 Difference between groups and conditions of particle counts by PAS (by ANOVA)

PAS	Mean* <i>Alternaria</i> spores/hr (95% CI)	P-value	Mean* total particles/hr (95% CI)	P-value
Children	5.5 (4.1,7.1)	0.96	9.1 (6.7,11.8)	0.81
Adults	5.5 (3.7,7.7)		9.2 (6.0,13.1)	
Indoors	3.6 (2.6,4.7)	0.0002	6.2 (4.4,8.1)	0.004
Outdoors	7.9 (5.6,10.6)		12.6 (8.5,17.5)	
Passive	3.2 (2.4,4.0)	0.0001	5.4 (4.1,6.9)	0.0001
Active	8.6 (6.1,11.5)		13.8 (9.4,19.1)	

*Means were calculated on square root transformed values and the results were back-transformed to the original scale of measurement.

There were moderate within-house correlations for the indoor passive condition using NAS and for the outdoor passive condition using PAS.

The overall correlation between *Alternaria* spore counts by NAS and PAS, adjusted for location and level of activity, was 0.27 ($P=0.003$). The relation of the difference between NAS and PAS and the overall level of the count (i.e. the mean) is shown in Fig. 2. The average ratio of NAS counts to PAS counts was 1.315. In other words NAS counts were, on average 31.5% higher than PAS counts for *Alternaria*. NAS was more sensitive for the detection of grass pollen particles: 58% of NAS had detectable grass pollen compared with 27.5% of PAS ($P<0.01$).

Factors affecting exposure levels

No significant difference was found between the numbers of particles collected by children compared to adults (Table 2 and Table 3). However, the location and level of activity both had significant effects on particle counts for the NAS and the PAS. Both moderate activity and an outdoor location were associated with higher *Alternaria* and total particle counts than low level activity and indoor location, respectively.

Discussion

This is the first study to quantify the levels of spores and pollen which are actually inhaled during normal daily activities. It found large variation in personal exposure even within family groups assessed within the same local environment. In general, the extent of exposure was strongly determined by the level of personal activity, was higher outdoors than indoors and was not related to the subject's age.

The significant differences between family members reflect the differences in activities, location within the home and dust raising of each subject, even though the members performed the sampling simultaneously in the same house. The differences between members of the same family were almost as great as, and in some cases greater than, differences between families.

As sources of pollen and fungi in this environment are outdoors, it was not surprising that exposure levels were higher when subjects were assessed outside the house. However, it is notable that substantial exposure to these particles did occur during period of moderate activity undertaken indoors. Given the much higher proportion of time that people spend indoors, it can be speculated that greater total exposure to outdoor allergens such as pollen may occur indoors than outdoors.

This paper demonstrates the increase in exposure to fungi and pollen which occurs with moderate increases in activity. Since the difference was observed with the PAS, for which collection rates are independent of minute ventilation, as well as the NAS, it is likely that this increased exposure is attributable to disturbance of particles rather than exercise *per se*.

In this study exposure did not differ between adults and children. This implies that, as noted above, levels of exposure to inhaled particles may not be substantially influenced by lung volume. Perhaps the difference in lung volume of children compared to adults is counterbalanced by their increased activity, and thereby minute ventilation and dust disturbance.

Relatively low correlation between the NAS and PAS counts was observed. Impaction and filter-type devices sample mixed populations of particles from the ambient air with different efficiencies [12,13]. NAS are single slot impaction samplers where the true inhaled dose enters the sampler but may not all be collected onto the sampling surface. This is due to the effect of the particles' aerodynamic size on the sampler's collection efficiency. Conversely, all particles entering a PAS are collected onto the sampling surface, minus loss to walls. The collection efficiency of PAS for mixed particles from ambient is variable [13], and may not reflect the proportions of different sized particles which would enter the nose.

Substantial variation has been demonstrated between simultaneous measures of the same environment. Renstrom found 22.9% variation between pairs of simultaneous PAS samples which were sampled close together on a sampling rig [2]. Difference have also been detected between levels collected with PAS worn at each lapel [13]. It is also possible that in our study, the low observed correlation is partly attributable to real differences in exposure between the lapel (where the PAS was worn) and the nose.

Direct and individual comparison of either form of personal sampling with Burkard counts was not attempted in detail as such a comparison would involve relating the times of all activities to the corresponding position of deposition on the Burkard tape. Nonetheless, the average outdoor PAS count of 99.7 *Alternaria* spores/m³ was similar to counts collected by the Burkard trap (59–197 spores/m³) over this period.

Previous attempts to measure personal exposure to outdoor bioaerosols are not common and have generally involved measurements with a single individual. The first study using inhalation air sampling was conducted by Blackley more than a century ago using an impaction device shaped like an upturned tobacco pipe held in the mouth [14]. More recently Morrow-Brown reported using an anaesthetic face mask coupled through a flow device to a large impaction collector to collect inhaled

particles in a pollinating grass field [15]. Leuschner and Boehm used a 'personal sampler' consisting of sticky microscope slide, worn on the lapel, to estimate exposure to pollen [1]. The latter reported substantial variation in counts between two people, neither of which related to Burkard measurements.

Other studies have used PAS to examine *Alternaria*, *Cladosporium*, total fungi and pollen levels in environments, generally where high exposures were expected. A small study of municipal lawn cutters ($n = 7$, one person per day) showed fungal spore levels similar to those described here [16], whereas in a study during wood fuel chip handling in an enclosed environment exposures were very high, ranging from 10⁶ to 10⁹ spores/m³ [17]. Of our six subjects with *Alternaria* NAS counts higher than 100 spores/hr outdoors, four were mowing lawn or in the same yard as the mowing, one was moving old wood in a dusty shed and one was raking a gravel driveway near the dusty shed's open doors.

Closing comments

This study highlights the very local nature of individual exposure to airborne allergenic particles. Attempts to explain day to day symptom fluctuations on the basis of ambient monitoring would be confounded by such individual variations. It also demonstrates that the cumulative level of indoor exposure to so called 'outdoor allergens' may be high and is related to activity disturbing dust reservoirs. Such exposure may be amenable to allergen avoidance interventions.

Acknowledgements

The authors would like to thank the NH & MRC for their funding support.

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