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MEASUREMENT OF PERSONAL EXPOSURE TO OUTDOOR AEROMYCOTA IN NORTHERN NEW SOUTH WALES, AUSTRALIA

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Abstract: Aerobiological sampling traditionally uses a volumetric spore trap located in a fixed position to estimate personal exposure to airborne fungi. In this study, the number and identity of fungi inhaled by human subjects (n=34), wearing Intra-nasal air samplers (INASs), was measured over 2-hour periods in an outdoor community setting, and compared to fungal counts made with a Burkard spore trap and Institute of Occupational Medicine personal filter air samplers (IOMs). All sampling devices were in close proximity and located in an outdoor environment in Casino, northern New South Wales, Australia. Using INASs, the most prevalent fungi inhaled belonged to soil or vegetation borne spores of *Alternaria*, *Arthrinium*, *Bipolaris*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Exserohilum*, *Fusarium*, *Pithomyces*, *Spegazzinia* and *Tetraploa* species, Xylariaceae ascospores, in addition to hyphal fragments. These results showed that inhaled fungal exposure in most people varied in a 2-fold range with 10-fold outliers. In addition, the INASs and personal air filters agreed more with each other than with Burkard spore trap counts ($r=0.74$, $p<0.0001$). These findings further support a new paradigm of personal fungal exposure, which implicates the inhalation of a spectrum of fungi more closely associated with soil or vegetation borne mycoflora and hyphal fragments than what is collected by stationary spore traps in the same geographic region.

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INTRODUCTION

Fungi are a diverse lineage of spore-bearing microorganisms that are ubiquitous bioaerosols in indoor and outdoor environments. Currently, 69,000 species have been identified out of 1.5 million estimated to exist [27]. To date, more than 80 fungal species have been identified to induce IgE mediated hypersensitivity [16, 26]. The airborne spores, hyphae and fragments of these fungi, are the principal dispersal agents, many of which are small enough to be inhaled into the lower airways [20, 22, 34]. Personal exposure to these fungal propagules, in particular *Alternaria* spores, are widely recognized to be risk factors for allergic rhinitis [5, 28], asthma [15, 48] and even respiratory arrest [35, 46]. However, the numbers of ambient spores that are required to elicit an adverse health effect is contentious and varies on an individual basis [30]. Industrial hygiene guidelines developed by the

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United States Occupational Safety and Health Administration have set a value of 1,000 cfus per m^{-3} or greater as unacceptable levels of fungal contamination [1]. Nonetheless, the enumeration and measurement of airborne fungi is highly dependent on the methods of sampling and analysis applied and, as such, airborne counts are often difficult to standardise and interpret. Additional variations arise from temporal, spatial and geographic variables [17, 41].

The benchmark for outdoor aerobiological sampling is the Hirst (Burkard) volumetric spore trap [25]. This is usually used while located in a fixed position, such as on the roof of a building. The trap operates by drawing in ambient air through an orifice at a constant rate (10 l/min) and deposits the airborne particles onto an adhesive tape, which is fixed to a rotating drum. The particles are then resolved by light microscopy and subjectively identified. The counts are used to estimate the level of exposure to airborne fungal spores in the region surrounding the spore trap. Similar devices, which correlate with Burkard counts and are based on similar air sampling principles have been developed and utilized in a number of outdoor exposure investigations; these include the Rotorod [7, 18] and liquid impinger [8] air samplers. More recently, the development of personal air samplers has enabled the measurement of personal exposure to fungal bioaerosols in occupational environments. Such personal samplers consist of a small filter membrane held in a sampling head and connected to a portable air pump. The filter head is generally worn on the lapel of an individual, so that it samples from the breathing zone. An example is the IOM personal aerosol sampler (SKC Inc, USA). The pump draws in the surrounding air at 2 l/min and has been shown in field trials to represent the inhalable fraction [31]. Alternative personal air sampling devices have also been developed and evaluated against benchmark aerobiological air sampling techniques [2-4]. However, variations between collection efficiencies of these mechanical samplers and differences in sampling strategies make it difficult to interpret personal exposure to airborne fungi as well, as compare studies. To date, there is no validated and accurate 'golden standard' of personal exposure, although measurements made with IOM samplers are often seen as the closest approximation to this.

Intra-nasal air samplers (INASs) are worn in the nose and use impaction to collect inhaled particles [21]. The INASs have high collection efficiency for larger particles (>5 µm in diameter), low air flow resistance and do not clog [21]. The INASs have been used to accurately measure individual personal exposure to a range of aeroallergen sources, including house dust mite [38], cat [13], cockroach [14], latex [37], rat [40] pollen and larger fungal spores [34]. These samplers provide novel insight into the quantity and distribution of inhaled particles as well as the variation in exposure between individuals. The aim of this study was to simultaneously measure exposure to fungal spores in an outdoor community setting with a Burkard spore trap, IOM personal aerosol samplers and INASs to qualitatively compare between the three different sampling methods. Variations between individual exposure patterns in the same location and at the same time were additionally investigated.

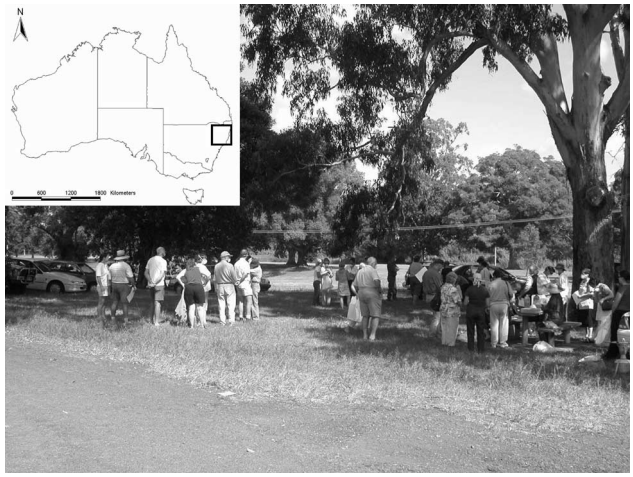

Figure 1. Sampling site and surrounding vegetation with subjects participating in the study on SaD 1; inset map of Australia depicting the geographical position of Casino, northern New South Wales and the (□) sampling region.

MATERIALS AND METHODS

Study location. The study was carried out in a public park in the town of Casino (Fig. 1) in northern New South Wales, Australia (28º52'S 153º03'E). The climate of Casino is classified as subtropical with an average yearly mean temperature of 19ºC and an average yearly rainfall of 110 cm (Bureau of Meteorology, NSW, Australia). The major vegetation formation of the trapping site consisted of several acres of open Eucalyptus woodland with an understorey of grass and herbaceous species, including *Paspalum notatum*, *Cynodon dactylon* and *Ambrosia artemisiifolia* (Fig. 1). Surrounding creeks and rivers are vegetated by riparian plant communities and residential and industrial areas have numerous representatives of exotic taxa [9].

Subjects. The samples in this study were collected as part of a concurrent study where the INASs were being assessed as a personal nasal filter, and information pertaining to the recruitment, age and clinical symptomology of the subjects has been published elsewhere [36]. The Human Ethics Committee of the Northern Rivers Area Health Service gave approval for the study and written informed consent was obtained from all subjects.

Study design. The study occurred on two consecutive Sundays (7 and 14 April 2002) during autumn, and subjects were asked to attend on both sampling days (SaD). On each SaD, subjects assembled at the study site at approximately 10:00 and were randomly allocated into groups of 8-10. Random allocation to each group was based on arrival time at the study site. Each group was then randomly allocated to receive active or placebo

INASs after baseline measurements for a concurrent study [36]. The subjects then placed the INAS into their nostrils. Subjects were asked to breathe through the nose for the 2-hour duration of the study, while remaining in their groups and engaging in only mild activity (sitting, walking and eating) in a central location in the park (Fig. 1). On each SaD, the meteorological parameters characterizing the study site consisted of warm and dry conditions with no wind; however, precipitation was recorded throughout the week prior to SaD 2.

Fungal exposure measurements. Ambient fungal spore levels were measured by 3 varying methods of analysis. A Burkard 7-day volumetric spore trap (Burkard Manufacturing Co., Rickmansworth, UK), located 3.5 m above the ground on the roof of a sports pavilion, operated continuously at the study site from 14 March-25 May 2002. The sampler was located less than 100 m away from where people were performing the measurements of personal exposure. The trap was calibrated to sample air at 10 l/min and the atmospheric particulate matter was deposited onto Melanex tapes coated with a thin film of Vaseline/paraffin/toluene mixture. The 7-day tapes were prepared as 24 h sections, and then mounted onto microscope slides and stained with Carberla's solution to enumerate pollen and fungal spores [44]. Data from the portion of tape corresponding from 10:00-12:00 was used. The fungal spores and hyphae were counted using light microscopy at a magnification of × 200 and expressed as the number of spores per 2 h.

On each SaD, 4 and 6 subjects (day 1 and 2, respectively) concurrently wore IOM personal samplers (SKC Inc, USA). These were run at 2 l/min and IOM sampling heads (Institute of Occupational Medicine, Edinburgh, UK) contained a Millipore BVXA (Millipore, Bedford, MA) membrane. The filters were removed from the heads and the collected fungal propagules were resolved using light microscopy as described above. The total fungal count was expressed as the number of spores per 2 h.

Inhaled fungal exposure was also measured for each SaD using the INAS. For counting, the adhesive core of the active filter was removed and Carberla's stain was placed directly onto the adhesive core and examined as described above. The fungal spores and hyphae were counted using light microscopy at a magnification of × 200; the left and right INAS adhesive cores were added and the total fungal counts expressed as the number of spores per 2 h.

Intra-nasal air samplers. The INASs, which were also evaluated as prophylactic filters in a concurrent study, are shown in Figure 2. The outer surface is made of a soft medical grade silicone and the inner polypropylene core was coated with Vaseline (Lever Rexona, North Rocks, Australia). The airflow through the device is non-linear and inhaled particles tend to remain in a linear trajectory and impact onto the adhesive surface. To accommodate different sized noses, 2 sizes of the INAS were used, with the appropriate size being allocated on the basis of external nasal appearance. INASs were kept in a sealed container until the time of their issue to study participants.

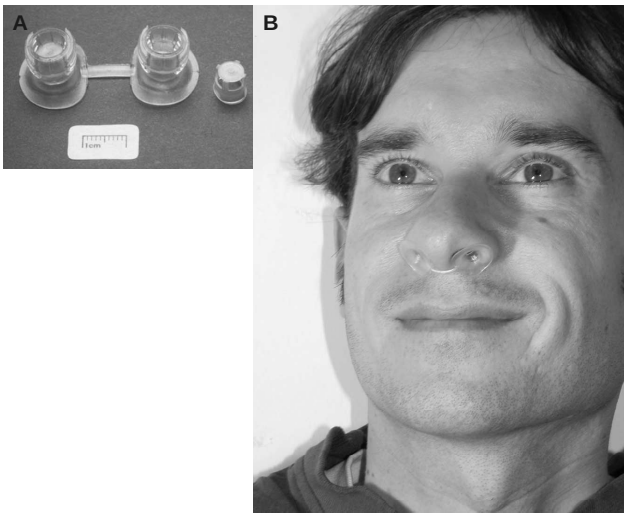

Figure 2. (A) Intra-nasal air sampler. Disassembled components for the intra-nasal air sampler, a soft silicon strap spans the septum of the nose and connects the two silicon frames that house the collection cups. (B) Fully assembled intra-nasal air sampler worn by a subject.

Capture efficiency of the INASs was established in an airflow rig. Rye grass (*Lolium perenne*), Ragweed (*Ambrosia artemisiifolia*), Bermuda (*Cynodon dactylon*) and Bahia (*Paspalum notatum*) grass pollen (Greer Laboratories Inc., Lenoir, NC) were aerosolised using a medical powder blower (Professional Medical Products, Greenwood, SC). Aerosol laden air was drawn in a steady flow from a plenum through the nasal filter and a downstream Millipore RAWG filter at flow rates spanning the normal human inspiratory flow range (4.6, 10.3, 21.7 and 32.5 l/min). Pollen grains were counted using an Olympus BX60 fluorescent microscope and capture efficiency was calculated as the number of particles collected by the nasal filter divided by the sum of particles collected by the nasal filter and the downstream membrane filter. Airflow resistance of the INASs was 4.1 cm H_2O/l/sec and was measured as previously described [21].

Statistical analysis. All data are reported as total fungal counts per 2 h period and the results expressed as medians and 25th and 75th percentiles, which were analyzed using Graphpad prism software (Prism 4, Graphpad, San Diego, CA). Since values were non-normally distributed the values for total fungal counts were log10 transformed for all individual genera counts and all absolute counts were log10 transformed plus 0.5, to account for the high number of zero values. Overall differences between the total counts of individual fungal genera for each SaD and the total left and right nostril fungal counts were examined by 1-way analysis of variance (ANOVA). The association between the INAS and IOM was calculated using the Pearson correlation coefficient. Statistical significance was defined for all tests as $p < 0.05$.

Table 1. Median, 25th and 75th percentile values of the total number of airborne fungi measured by three different air sampling methods in an outdoor community setting.

	Method of Analysis			Method of Analysis		
Fungal genera	Burkard[a]	IOM[b]	INAS[c]	Burkard	IOM	INAS
	Sampling Day 1			Sampling Day 2		
Fungal spores ≥ 10 μm						
Acrodictys	3	0 (0-0)	0 (0-0)	20	0 (0-0)	0 (0-0)
Alternaria	18	64 (42-80)	34 (23-87)	8	48 (21-80)	128 (76-228)†
Ascobolus	9	0 (0-0)	0 (0-0)	100	0 (0-0)	0 (0-0)
Beltrania	27	0 (0-4)	0 (0-1)	16	0 (0-0)	0 (0-0)†
Bipolaris	12	16 (12-18)	5 (1-13)	4	200 (0-369)	108 (56-246)†
Cerebella	0	0 (0-0)	0 (0-0)	0	0 (0-0)	0 (0-0)
Curvularia	45	176 (172-228)	160 (116-224)	48	304 (296-352)	196 (96-266)
Dictyosporium	0	0 (0-0)	0 (0-0)	12	0 (0-0)	0 (0-0)
Epicoccum	18	8 (8-14)	47 (25-64)	88	56 (16-80)	204 (102-290)†
Exserohilum	6	32 (20-36)	20 (9-35)	12	32 (5-56)	20 (16-30)
Fusariella	1	8 (8-8)	6 (4-10)	0	32 (24-32)	16 (8-28)
Fusarium	0	8 (8-12)	0 (0-3)	0	0 (0-8)	12 (5-38)†
Hyphal fragments	30	656 (624-692)	250 (170-357)	8	944 (656-1024)	232 (142-356)
Isthmospora	0	0 (0-0)	0 (0-0)	0	0 (0-0)	0 (0-0)
Leptosphaeria	108	8 (8-8)	8 (5-12)	4	0 (0-0)	4 (0-8)†
Leptosphaerulina	27	0 (0-0)	0 (0-0)	140	0 (0-0)	0 (0-0)
Pithomyces	2	64 (42-116)	99 (49-148)	20	32 (24-40)	52 (32-72)†
Pleospora	1	0 (0-0)	0 (0-0)	0	0 (0-0)	0 (0-4)
Rust	0	0 (0-0)	0 (0-0)	0	0 (0-0)	0 (0-0)
Spondylocladiella	27	56 (43-64)	4 (0-13)	24	28 (24-40)	44 (24-62)†
Sporidesmium	0	8 (4-8)	0 (0-0)	0	0 (0-0)	0 (0-0)
Stemphylium	0	0 (0-0)	0 (0-0)	0	0 (0-0)	0 (0-0)†
Spegazzinia	6	96 (80-104)	47 (31-79)	8	16 (16-21)	16 (12-34)†
Tetraploa	2	8 (8-12)	16 (10-31)	0	0 (0-8)	16 (4-24)†
Ulocladium	0	0 (0-0)	0 (0-1)	0	0 (0-0)	0 (0-4)
Xylariaceae	195	1056 (720-1080)	697 (402-1093)	112	968 (688-984)	372 (274-552)†
Fungal spores 3-10 μm						
Amphisphaeria	0	0 (0-0)	0 (0-0)	0	0 (0-0)	0 (0-0)
Arthrinium	20	1312 (1004-5724)	410 (194-781)	8	120 (104-128)	108 (62-166)†
Basidiomycete	3	8 (5-14)	11 (1-16)	0	8 (0-8)	8 (4-18)
Botrytis	0	0 (0-0)	0 (0-0)	16	0 (0-0)	0 (0-0)
Cladosporium	3495	1048 (968-1256)	42 (32-85)	3528	4970 (4578-5180)	32 (8-106)†
Coprinus	57	0 (0-0)	0 (0-0)	440	0 (0-0)	0 (0-0)
Delitschia	0	0 (0-0)	0 (0-0)	0	0 (0-0)	0 (0-0)†
Myxomycete	0	0 (0-0)	0 (0-0)	28	0 (0-0)	0 (0-0)
Smut	0	0 (0-0)	0 (0-0)	0	0 (0-0)	0 (0-0)
Sporomiella	21	0 (0-0)	0 (0-0)	118	0 (0-0)	0 (0-0)
Torula	0	0 (0-0)	2 (0-5)	0	0 (0-0)	0 (0-0)†
Fungal spores ≤ 3 μm						
Aspergillus-Penicillium	528	0 (0-0)	0 (0-0)	96	0 (0-0)	0 (0-0)
Unknown	591	16 (12-18)	78 (36-138)	356	40 (24-40)	36 (20-66)
Total	5202	4794 (4718-8642)	2593 (1749-3663)	5214	7522 (7521-8300)	1808 (1284-2358)†

[a]Burkard counts are representative of only one observation (n=1); [b]IOM spore counts include Median, 25th and 75th percentiles (n=4 SaD 1 and n=6 SaD 2); [c]INAS spore counts represent the median (25th and 75th percentiles) number of spores inhaled by each subject (n=34 SaD 1 and n=31 SaD 2). The total spore count for each genus is derived from the addition of left and right INAS nostril collection cups; †Denotes the level of significance of ANOVA statistical analyses between SaDs 1 and 2 INAS spore counts, which were log transformed to normalise the data. † $p<0.05$.

RESULTS

The median and 25th and 75th percentile values of airborne fungi per 2 h measured by each of the 3 sampling methodologies are presented in Table 1. The static Burkard spore trap measurements consisted of only 1 observation and there were a total of 25 genera collected on both SaD 1 and SaD 2 (Fig. 3). The most frequent fungi collected on SaD 1 included *Cladosporium*, *Aspergillus-Penicillium* and *Leptosphaeria* species, Xylariaceae ascospores with smaller concentrations of *Coprinus*, *Curvularia* and hyphal fragments encountered.

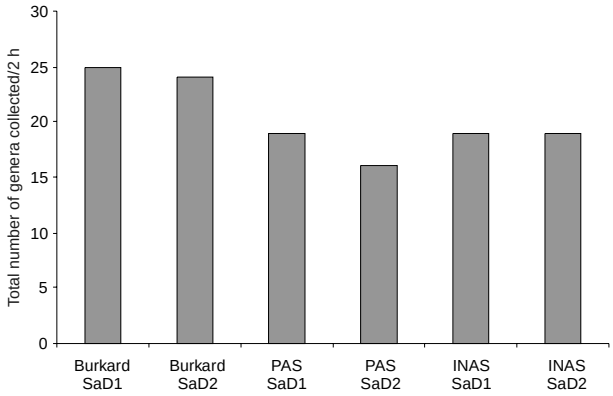

Figure 3. Total number of fungal genera collected by each air sampling technique on each sampling day.

IOM and INAS devices showed a similar, although smaller spectrum of airborne fungal genera compared to the Burkard spore trap (Fig. 3). The total spore numbers per 2 h for the Burkard spore trap were higher than IOM and INAS for several smaller-spored fungi, including *Aspergillus-Penicillium*, *Cladosporium*, *Sporomiella*, *Coprinus* and *Leptosphaerulina* species. However, a number of other fungi were less frequently collected on the Burkard compared to IOM and INAS, for example, *Alternaria*, *Curvularia*, *Exserohilum*, *Spegazzinia* and *Arthrinium* species, Xylariaceae ascospores, in addition to hyphal fragments (Tab. 1). Most of the latter are larger-spored species.

The spectrum of fungal genera and the total counts collected by each method of analysis on SaD 2 qualitatively differed compared to SaD 1. The Burkard spore trap collected similar quantities of *Cladosporium*, *Curvularia* and Xylariaceae ascospores on both days; however, greater total numbers of *Ascobolus*, *Coprinus*, *Leptosphaerulina*, *Pithomyces* and *Sporomiella* species were collected on SaD 2. The number of genera collected on the IOMs was less on SaD 2, while the numbers of genera collected by INAS remained unchanged (Fig. 3). As in SaD 1, greater airborne counts of fungal spores >10 μm in diameter were collected by both IOMs and INASs, compared to Burkard measurements. The most frequent taxa were represented by *Cladosporium*, *Curvularia*, *Bipolaris*, *Epicoccum* and *Spegazzinia* species, ascospores belonging to the Xylariaceae, in addition to hyphal fragments. Other fungi collected by IOM and INAS and not by the Burkard spore trap included Basidiomycete, *Fusariella*, *Fusarium* and *Tetraploa* species (Tab. 1). For both SaDs, the mean proportion of unknown fungal spores accounted for 6.5% of the total count for the Burkard spore trap, 0.4% for IOM and 2.5% for INAS.

The total number of fungi collected by each sampling method varied between each SaD (Tab. 1) with INAS spore counts significantly higher on SaD 2 compared to SaD 1 ($p<0.05$). On an individual genera basis, the overall variations between INAS fungal counts for each SaD were heterogeneous (Tab. 1). Spore counts measured by INASs were significantly higher on SaD 1 ($p<0.05$) for

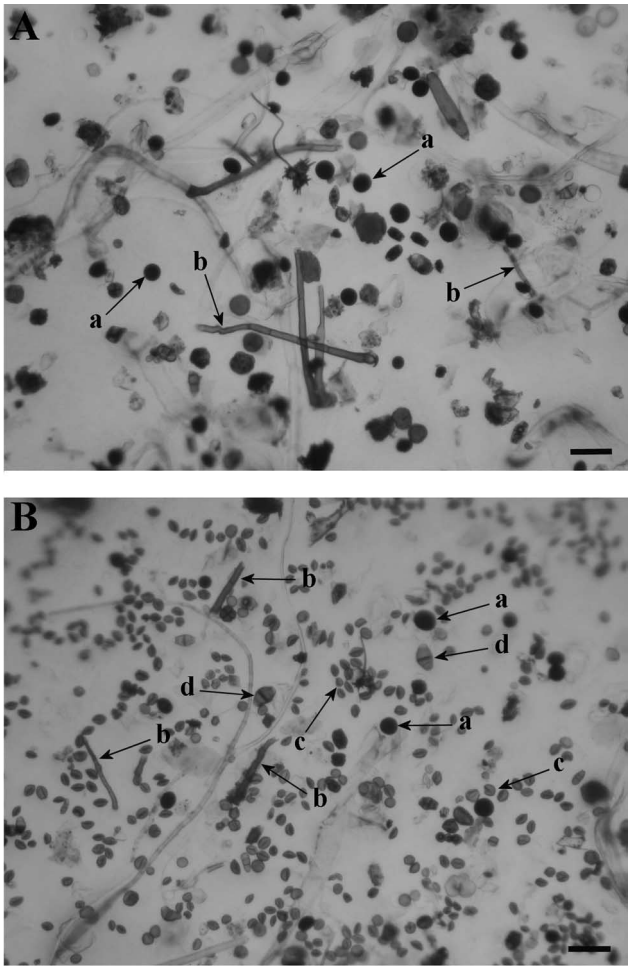

Figure 4. Photomicrographs of collected fungal conidia and hyphal fragments. A. Ascospores belonging to Xylariaceae (arrow a) and hyphal fragments (arrow b). B. fungal spores of *Arthrinium* (arrow c) and *Curvularia* (arrow d) species. Scale bar, 20 μm.

the fungal species *Tetraploa*, *Pithomyces*, *Arthrinium*, *Stemphylium*, *Torula*, *Beltrania*, *Cladosporium*, *Leptosphaeria*, *Delitschia* and Xylariaceae ascospores and significantly higher on SaD 2 for *Alternaria*, *Bipolaris*, *Fusariella*, *Epicoccum*, *Spondylocladiella* and *Fusarium* species ($p<0.05$). No significant differences were found between spore counts for SaDs 1 and 2 for species belonging to *Curvularia*, *Exserohilum*, *Acrodictys*, *Isthmospora*, Smut, Rust, Basidiomycetes, *Ulocladium* and *Leptosphaerulina*, in addition to hyphal fragments (Tab. 1).

The total number of spores belonging to the 5 most frequent taxa that were inhaled by each subject on each SaD is presented in Figure 5. These results show that inhaled exposure in most people varied within a 2-fold range with 10-fold outliers. Photomicrographs of the most common taxa collected by INASs are also presented in Figure 4. Furthermore, the comparison between the numbers of spores belonging to the 9 most frequently recorded genera that were captured on left and right nostril INAS collection cups is shown in Figure 6. No significant difference between left and right nostrils was observed for any of the fungal genera ($p>0.05$).

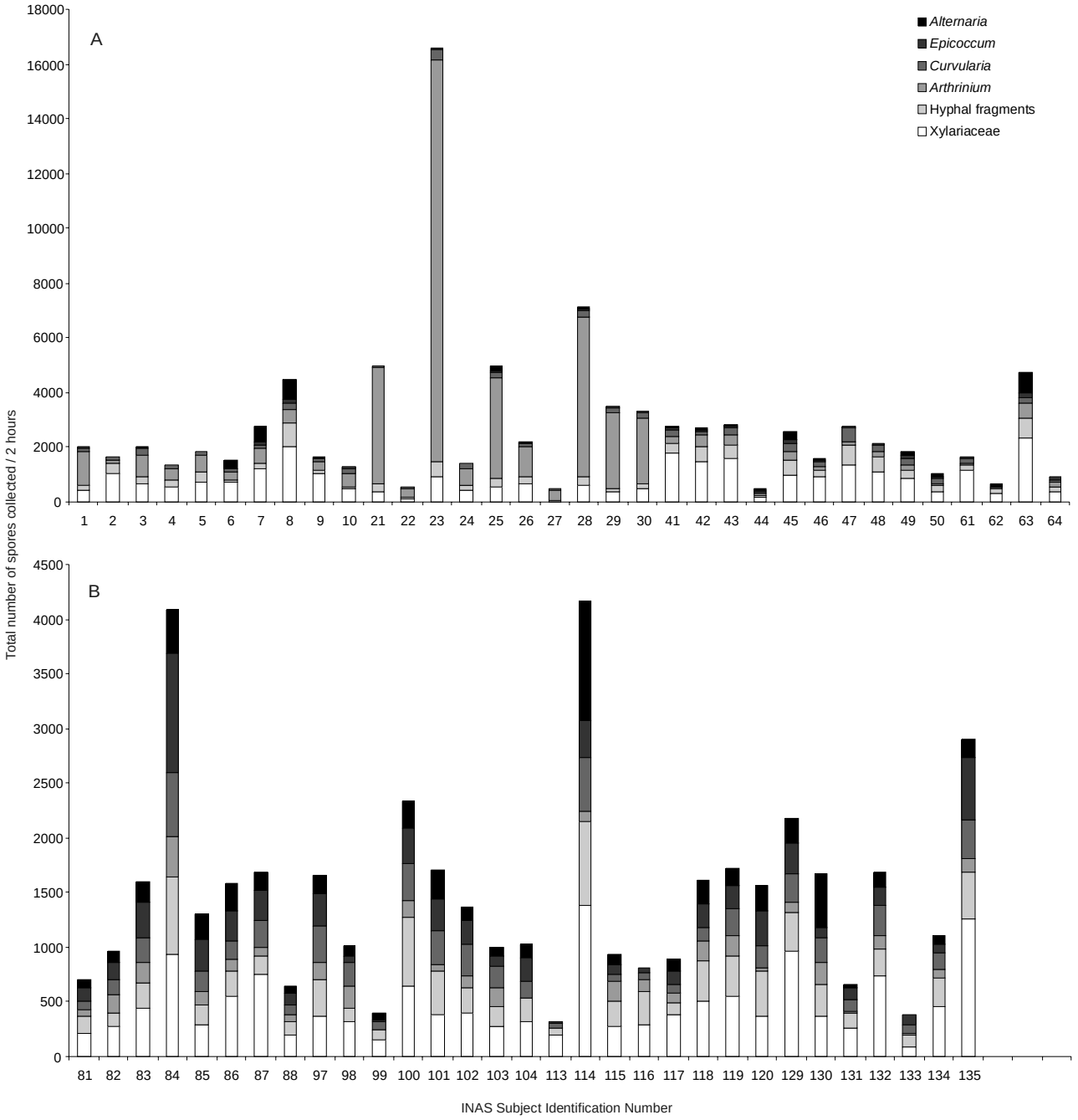

Figure 5. Relative abundance of fungal spores belonging to the five most frequent genera inhaled by each subject. A. Sampling Day 1. B. Sampling Day 2. Note scale differences between each sampling day.

Pearson correlation co-efficients between fungal genera counts for IOM and INAS samplers are presented in Figure 7. The number of inhaled airborne spores and hyphae collected by INASs was highly associated with those counts collected on IOM air samples ($r=0.74$, $p<0.0001$).

DISCUSSION

This is the first study to quantify both the numbers and genera of fungi that are actually inhaled during normal outdoor activities. Furthermore, this study provides novel insight into the variations between traditional measurement techniques, and the number and types of fungi inhaled between subjects in the same location.

Overall, we have shown that there was a wide (~10×) fold range in the total number of fungal particles inhaled by subjects located in the same geographic location. The variability between subjects probably reflects combined differences in respiratory patterns, the distribution of fungi within the local microenvironment and variations in subject activity, which affect the disturbance of spores by individuals. Previous studies investigating the inhalation of *Alternaria* and *Cladosporium* spores using INASs,

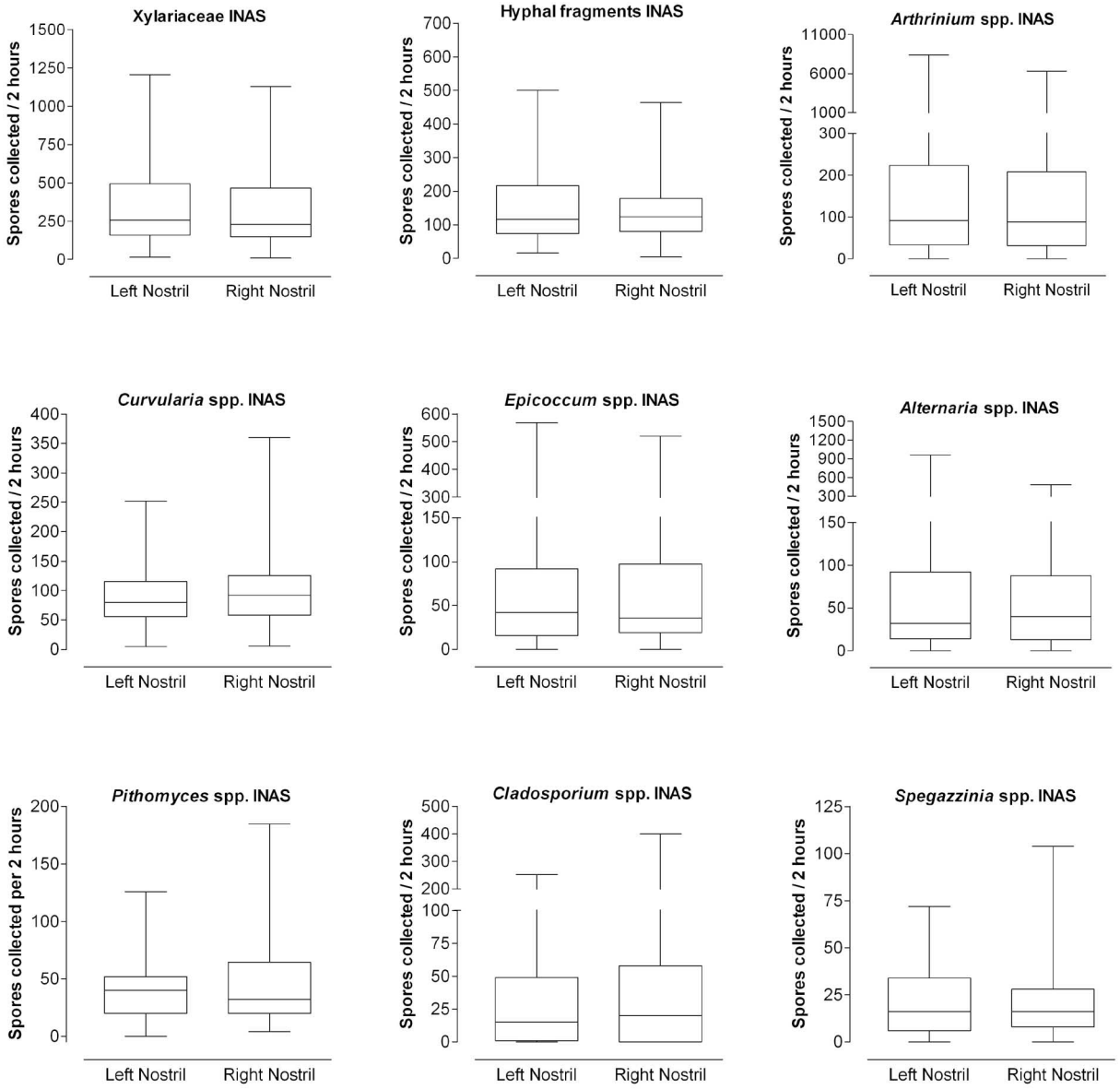

Figure 6. Comparison between the relative abundance of total fungal spores collected by left and right nostril INAS collection cups for the nine most frequent genera. Box plot denotes minimum, 25th percentile, median, 75th percentile and maximum values. No significant differences were observed between left and right nostrils for any of the fungal genera.

have shown that variations in spore levels within families of the same household were largely associated with the type and intensity of personal activity [34]. Similarly, the dispersal of fungal spores from various flooring materials has been shown to be greatest following disturbances either by walking or vacuuming [12]. Personal activity in this study was restricted to mild daily events, including sitting on the ground and walking within a 100 × 100 m area; however, the differences such events might have on personal exposure has not been explored.

The requirements for fungal growth and reproduction include the breakdown of living or decaying plant material, which restrict many genera to the uppermost soil horizon, the organic layer. Microscopic examination of soil has shown that fungi are present both as spores and hyphae in the O, A, B and C soil horizons with the highest concentrations restricted to highly organic layers [47]. The fungal genera most commonly isolated from the soil include *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Epicoccum* and Xylariaceae species [6, 23, 43, 45, 47]. It has been proposed that disturbances to the surface O Horizon result in the detachment of spores and fragmentation of hyphae from the mycelium, and also with the aid of wind and other atmospheric vectors; these fungal particles may aerosolize directly into the inhalable zone of the subject [34]. This is well-illustrated on an environmentally larger disturbance scale, where cropping activities of wheat have been shown to significantly increase the airborne concentration of *Alternaria* in rural environments [32]. Thus, the collection of larger concentrations of fungi associated with soil by INAS, in addition to high concentrations of hyphal fragments, might have

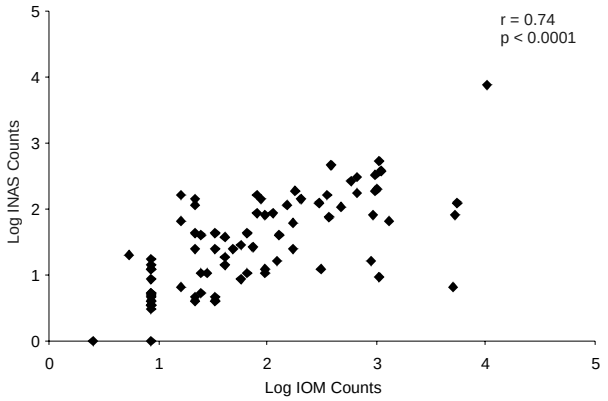

Figure 7. Correlation between IOM and INAS total fungal counts. In the figure: r = Pearson rank correlation co-efficient. Correlations are significant at 0.05 level.

resulted from the light personal activity engaged by the subjects.

This paper demonstrates that the detection of variability between collected spore numbers on different air sampling days was dependant on both the sampling methods and genera counted. Airborne fungal counts are well-recognized to fluctuate between spatial, temporal and geographic parameters, as well as between the methods of air sampling [17, 41]. Regional airborne counts are largely dependent on the prevailing weather conditions, including temperature, humidity and wind direction of the surrounding environment, in addition to the pre-existing substratum nutrient conditions, which facilitate the production and release of fungal spores. Disturbing this subtle balance alters the growth, reproduction and aerosolization of fungal propagules from fungal colonies, thus modifying daily concentrations. Previous studies have demonstrated that the spores of specific fungal genera in some cases require moisture in order to disseminate the spores, whereas other fungi require dry conditions [11]. This was particularly evident on the first SaD when the study location was characterized by dry environmental conditions, which facilitated higher airborne spore counts of *Arthrinium*, *Spegazzinia* and Xylariaceae species. However, pre-sampling precipitation on SaD 2 favoured the release of *Epicoccum*, *Bipolaris* and *Curvularia* spores.

The variations observed in the number and spectrum of fungal spores collected by each method of analysis can be attributed to several parameters associated with the operation, collection and quantification of each air-sampling device. For instance, the Burkard volumetric spore trap is a static air-sampling device generally placed at ground level or on top of a building. The local topography, environment and orientation of the air sampler has been shown in numerous studies to yield a wide spectrum of fungi, in addition to total fungal concentrations [11, 29, 42]. The collection of airborne fungal particles by a spore trap is influenced by a combination of localized and more distant sources, and may not accurately represent the distribution of particles in a neighboring geographic locale [33]. The height at which a sampler is placed is likely to also influence the final outcomes of a study. It has been shown that fungal spore concentrations decrease with increasing height, as demonstrated for *Alternaria* and other spore types [10, 24, 39]. Thus, spore concentrations are generally greatest at ground level and this was particularly reflected in the INAS and IOM spore counts reported in the present study.

To date, the INAS method is the closest air sampling technique available for a true assessment of personal exposure over short temporal intervals. The sampler has been shown to efficiently collect most spherical latex particles above 5 μm [21]; however, for smaller particles (<5 μm), including unicellular fungal conidia and hyphal fragments, the collection efficiency decreases. This is particularly evident when comparing Burkard, IOM and INAS samples. Our findings show that relatively low numbers of small fungal particles (<5 μm), in particular *Aspergillus-Penicillium* and *Cladosporium* spores, were collected on the INASs compared to IOM and Burkard counts. In some cases, the total numbers of fungi collected by IOM personal aerosol samplers were approximately 4-fold greater than that collected by INAS. These variations can be accounted for by limitations in the INAS collection efficiencies for smaller particles, larger sampling collection areas and by difficulties visualizing and subjectively identifying smaller spores collected on the opaque INAS impaction surface. Conversely, the Burkard spore trap has a much broader collection efficiency for airborne particles compared to the other devices, which is reflected by the diversity and size ranges of airborne fungal spores collected in the present study. In addition, smaller spores belonging to *Aspergillus-Penicillium* and *Cladosporium* species were much more easily resolved on the stained Burkard tape than the other methods; this is probably why smaller fungal spores, as well as some larger opaque spores, for example, *Leptosphaerulina* and *Aspergillus-Penicillium*, were only observed on the Burkard impaction surface. Therefore, to accurately monitor inhaled airborne exposure to fungi, it will be necessary to continue to develop INAS to collect particles down to 2 μm in diameter.

The results of this study also demonstrate for the first time the collection of inhaled fungal fragments and hyphae in an outdoor environment. These morphologically indiscernible particles have recently been shown to express detectable allergen in environmental samples detected with the Halogen immunoassay [22]. The proportion of inhaled hyphae confirms previous investigations that airborne fragments are often higher in concentration than the numbers of spores of a single fungal genus [19, 20, 22]. The importance of these airborne particles, often ignored in previous airborne studies, is slowly being recognized and the results of this study further demonstrate the significant concentration in which these particles are inhaled. Furthermore, the genera collected by the INASs provide an entirely different

spectrum of fungal exposure compared to those reported in previous studies. Although dependent on the geographic location and efficiency of the sampler, the dominant inhaled fungi included *Arthrinium*, *Curvularia*, *Epicoccum*, *Pithomyces*, *Spegazzinia*, *Bipolaris* and Xylariaceae species, which have all been recently shown to express detectable allergen in environmental samples [22]. These findings further support a new paradigm of personal fungal exposure, where a substantial proportion of the airborne fungal biomass rather than a limited group of genera contributes to the aeroallergen load.

CONCLUSIONS

The measurement of personal exposure to airborne fungi using INASs is a sensitive and unique technique compared to pre-existing methods of analysis. The collected fungal particles represented are a true reflection of what is actually being inhaled, although this is confounded by low collection efficiency for smaller particles. The results of this study show that inhaled fungal exposure, in most people in the same location, varied within a 2-fold range with 10-fold outliers. Comparatively, the INASs and IOM personal aerosol samplers were associated more to each other than to Burkard spore traps. The variations between each of the air sampling techniques investigated can be attributed to variables associated with the operation, collection and quantification of each air sampling device. Our results further demonstrate the significance of recently identified fungal allergen sources, including airborne hyphae and numerous other allergenic genera to personal exposure. These findings advance our understanding of personal exposure to airborne fungi; however, future investigations are required to improve diagnostic techniques, in addition to understanding the role of fungal allergens in allergic rhinitis and asthma.

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