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Do indoor plants contribute to the aeromycota in city buildings?

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Abstract Many studies have focused on the sources of fungal contamination in indoor spaces. Pathogenic fungi have been detected in the potting mix of indoor plants; however, it is unclear if plants in indoor work spaces make qualitative or quantitative contributions to the aeromycota within buildings. The current work represents a field study to determine, under realistic office conditions, whether indoor plants make a contribution to the airborne aeromycota. Fifty-five offices, within two buildings in Sydney's central business district, were studied over two seasonal periods: autumn and spring. We found that indoor plant presence made no significant difference to either indoor mould spore counts or their species composition. No seasonal differences occurred between autumn and spring samples. Indoor spore loads were significantly lower than outdoor levels, demonstrating the efficiency of the heating, ventilation and air conditioning systems in the buildings sampled. Neither the number of plants nor the species of plant used had an influence on spore loads; however, variations of those two variables offer potential for further studies. We conclude that conservative numbers of indoor plants make no substantial contribution to building occupants exposure to fungi.

Keywords Indoor air quality · Aeromycota · Indoor plants · Airborne fungi · Office buildings

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1 Introduction

Elevated airborne fungal spore concentrations when found indoors are of great concern, having been linked to health issues such as sick building syndrome (Takeda et al. 2009; Meyer et al. 2004) and fungal disease (Hunter et al. 1988; Staib et al. 1978). Adverse health effects caused by fungi result from the production of allergens, irritants and mycotoxins, eliciting symptoms of headache, coughing and dermatitis (Mendell et al. 2011). Serious fungal infections may result in immunocompromised individuals from exposure to certain opportunistic fungal species (Maschmeyer et al. 2007).

It has been proposed that indoor plants could act as a significant source of fungal inocula (Engelhart et al. 2009; Hedayati et al. 2004; Lass-Flörl et al. 2000; Staib et al. 1978, Summerbell et al. 1989), although no systematic study to date has tested this claim within air-conditioned office buildings.

Indoor relative humidity (RH) levels in excess of 80 % are thought to be the leading cause of fungal

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amplification (Adan and Samson 2011). Indoor air may vary in humidity due to numerous factors such as seasonal variability and building design; however, heating, ventilation and air conditioning (HVAC) systems moderate heat and moisture in buildings to produce environmental conditions such that indoor RH is generally maintained between 60 and 80 % (ASHRAE 1992).

Indoor airborne fungi have both indoor and outdoor sources (Chih-Shan and Hsu 1997), although modern mechanically ventilated buildings prevent the intrusion of a large proportion of outdoor fungal aerosols (Burge et al. 2000). In temperate climates such as Sydney, Australia, city buildings with central HVAC systems and low air exchange rates, generally have less than 200 fungal colony-forming units (cfu)/m^3 (Hess-Kosa 2011), compared to outdoor levels that routinely exceed 1,000 cfu/m^3 and may approach 10,000 cfu/m^3 during warmer, more humid months (ACGIH 1989).

In buildings considered acceptable for human occupation, the taxonomic diversity of indoor fungi should be broad and parallel that found outdoors (Gots et al. 2003). Factors that indicate potential fungal contamination in a building, or that environmental conditions exist that may favour fungal growth, include the amplification of one or two spore types to high concentrations, the presence of fungi that are not present in outdoor air and total indoor concentrations exceeding those outdoors (Horner et al. 2008). The main sources of filamentous fungi in indoor air are damp building materials, carpets and sometimes bathrooms and water supplies (Anaissie et al. 2002; Smith and Kagan 2005).

Humans inhale considerable quantities of fungal material during normal activities, especially from outdoor air, which reduces the quantitative influence of indoor air on total fungal exposure, as long as the indoor air contains less fungal material than outdoors (Madelin 1994). For immunocompetent individuals, pathological responses to airborne fungi are highly variable, depending on the allergen sensitivities of the individual (Green et al. 2003). It is widely accepted that fungal bioaerosols contribute to poor indoor air quality (ACGIH 1999).

Considerable research has investigated the point source for pathogenic fungi indoors, especially in hospitals, with some implicating indoor potted plants as a potential source (Engelhart et al. 2009; Hedayati et al. 2004; Lass-Flörl et al. 2000; Staib et al. 1978; Summerbell et al. 1989). As most fungi require moisture and are saprophytic, and indoor plants tend to require watering and contain dead organic matter in the potting mix, this hypothesis is plausible. However, the presence of pathogens in potting mix does not necessarily correlate with proportional increases in those taxa in airborne spore loads. Burge et al. (1982) concluded that modest numbers of indoor plants make no significant contribution to aeroallergen prevalence in homes, provided the plants were undisturbed. Further, Lehtonen et al. (1993) collected air samples whilst disturbing the indoor plants and found no marked changes in the spore concentration, in contrast to a similar study performed by Reponen et al. (1992). Thus, the function of indoor plants as substantial sources of indoor mould spores is cryptic and requires further research. Furthermore, no study has been conducted to determine the influence of indoor plants on the aeromycota in buildings with central HVAC systems.

The current work represents a field study to systematically test the hypothesis, under realistic office conditions, whether indoor plants make a quantitative and/or qualitative contribution to the airborne aeromycota. The specific project aims were to

1. determine the effect of indoor plants on humidity and temperature and to indicate whether they could be affecting indoor conditions so as to encourage indoor fungal growth;
2. determine whether indoor plants make a detectable contribution to the diversity and abundance of indoor fungal spore loads;
3. compare indoor airborne spore loads and fungal diversity to outdoor air;

2 Materials and methods

2.1 Buildings and offices

Two seven storey office buildings at the University of Technology, Sydney (UTS) in New South Wales, Australia, were used for the investigation. Both buildings are located in the Sydney CBD. Both have HVAC systems; one building is 16 years old and the other 4 years old and both accommodate a mixture of offices, lecture rooms and laboratories. Neither

building showed any evidence of dampness, and a consultation with the building services manager indicated no history of dampness or mould problems. The offices sampled were generally occupied by a single staff member. Office mean area was 16 ± 1.8 m^2; and volume 43 ± 2 m^3. The 55 offices sampled were selected by way of staff in the buildings being informed of the project and volunteering to participate. To increase the sample size of our study, time-for-space substitution was used: After completion of the first three-month sampling round (autumn samples), treatments were randomly reallocated to the same offices (spring samples).

2.2 Treatments

As it was predicted a priori that the surface area of the potting mix would have a stronger influence on airborne spore numbers than number of pots, varying numbers of pots of two diameters were used to modulate the pot surface area across the multiple plant treatments. Five experimental treatments were assigned at random to participant offices at 11 offices per treatment:

1 *S.w*: One 200-mm-diameter pot plant containing *Spathiphyllum wallisii* 'Petite' (Peace Lily); potting mix surface area 31,400 mm^2
3 *S.w*: Three 200-mm-diameter pots plants containing *Spathiphyllum wallisii* 'Petite' (Peace Lily); surface area 94,200 mm^2
1 *D.d*: One 300-mm pot containing *Dracaena deremensis* 'Janet Craig'; surface area 70,650 mm^2
2 *D.d*: Three 300-mm pots containing *Dracaena deremensis* 'Janet Craig'; surface area 94,200 mm^2
0 plants: Reference (control) group with no plants

Plant materials were supplied the National Interior Plantscape Association (NIPA), sourced from TLC Indoor Gardens, Sydney and containers from The Container Connection, Sydney. The potting mixtures were those supplied for the individual species by the plant industry. In each case, the mixture comprised a formulation based on a standard 'indoor' mixture of composted hardwood sawdust, bark fines and coarse river sand (approximately 2:2:1); with a 9-month slow-release comprehensive granulated fertiliser (Macrocote, Sydney, NSW). Self-watering pots were used for all pot sizes, and plants were watered and dead leaves removed weekly. Plants were maintained in a glasshouse for 6 weeks between test periods.

2.3 Air samples

Autumn samples were taken on 28 April, 19 May and 9 June 2008, and spring samples were taken on 8 September, 29 September and 20 October 2008. Air samples were taken when the plants were not disturbed. Samples were collected between 11 am and 1 pm.

Office temperatures and relative humidity (RH) were measured using a Portable IAQ-Calc Indoor Air Quality Meter (TSI Inc. MN, USA). Mould samples were collected using an Reuter Centrifugal air sampler (RCS; Biotest Diagnostics Corporation, Denville, New Jersey, USA), which operates on the principle of impacting particles from the sample air onto an agar medium coating a plastic backing strip by centrifugal force (Zhen et al. 2009). Sabouraud's dextrose agar (SDX; Biotest AG, Germany) was used as the growth medium, as its low pH (~ 5) is known to inhibit most bacterial growth. The SDX formulation used did not contain cycloheximide, as this is known to suppress the growth of some common fungi, including pathogens such as *A. fumigatus* (El-Ani 1975). The sampler was disinfected thoroughly with 70 % ethanol between samples. Office air samples were collected from approximately 50 cm above the surface of the desk, where the occupant would typically be working, to replicate as closely as possible the breathing space of the occupant. Outdoor samples were taken at the same time as the indoor samples: Four samples on each sampling interval were taken from sites proximal to the air conditioning intakes of each building. Two-minute (80 L) samples were collected from offices, and 30-s samples (20 L) from the air conditioner intakes, the smaller samples indicating the greater inoculum density detected in outdoor air samples. After collection, strips were aseptically removed from the RCS sampler, re-sealed in their plastic sleeves and incubated in the dark at 23 °C for 7 days. If colony development was inadequate after this period, strips were re-incubated until adequate colony growth occurred (up to 21 days), or until growth from other colonies became confluent. After incubation, the strips were stored at 4 °C to stop colony growth until identification was conducted (2–12 weeks).

Fungi were sampled from the strips using cellotape and stained with lactophenol cotton blue. Mould

colonies were identified to genus level using gross microscopic morphology, utilising the descriptions and keys of Alexopoulos et al. (1996), Ellis et al. (2007), Klich and Pitt (1988) and Mycology Online (2008–2012). Colonies that had not produced sufficient reproductive structures to allow for identification were subcultured onto potato dextrose agar (PDA; Oxoid) and SDX plates and re-incubated for up to 20 weeks. Fungi which did not produce fruiting bodies after 21 weeks of incubation, and were therefore unidentifiable, were recorded as sterile mycelia. All yeast incidences were pooled, as they were not the focus of this investigation.

2.4 Data analysis

Spore counts for the various treatment groups were expressed as mean colony-forming units per m^3 of sampled air (cfu/m^3). Univariate analyses were conducted using SPSS v20 (IBM Corp. 2011) and multivariate analysis using PRIMER v6.1.6 (Primer-E Ltd, 2006), except for canonical correspondence analysis, which was performed in CANOCO v4.51 (Biometris 2003). Data were transformed where necessary to improve homogeneity of variances. Average RH, temperature, spore numbers, number of fungal genera detected and indoor/outdoor ratios were compared using repeated measures general linear model ANOVAs, with the between subjects factors: treatment, season, and building, and the within-subjects factor; sampling time.

Fungal community differences between treatment groups were compared using analysis of similarities (ANOSIM), and where there were significant differences between groups, the fungal taxa that best differentiated between the groups were determined by similarity percentages analysis (SIMPER). To determine whether the differential abundance of fungal genera across the dataset was related to relative humidity, temperature or outdoor spore numbers, canonical correspondence analysis (CCA) was performed.

Statistical significance was tested at alpha = 0.05.

3 Results

Mean office temperatures varied significantly between seasons ($P = 0.000$), although the difference was small (autumn = 22.88 °C; spring = 22.47 °C). There was also a significant difference in mean RH between seasons ($P = 0.000$), with greater mean RH in autumn (56.96 %) than spring (46.29 %). The humidity levels detected ranged from 40.2 to 69.3 %, which lie generally within the optimal comfort range for building occupants of 40–60 % (ASHRAE 1992). There was no significant difference in temperature amongst plant treatment groups ($P = 0.080$), but RH varied significantly between planted and reference offices ($P = 0.008$). On average, plants increased indoor RH by 1.32 % over reference offices. Office conditions did not vary between the two buildings (RH; $P = 0.649$, temperature; $P = 0.059$).

Average numbers of cfu/m^3 detected in the treatment groups are shown in Fig. 1. Species composition for all treatments is shown in Tables 1 and 2.

There was no significant difference between the average numbers of spores detected in autumn and spring (Fig. 1; $P = 0.150$). Amongst the treatment groups, there were significantly greater average numbers of spores in the outdoor treatment group than in any of the indoor groups ($P = 0.000$), but there were no significant differences in the spore counts amongst indoor treatments. Spore numbers were also similar between the two buildings ($P = 0.843$). There was no significant correlation between average spore numbers and relative humidity ($P = 0.574$) or temperature ($P = 0.481$).

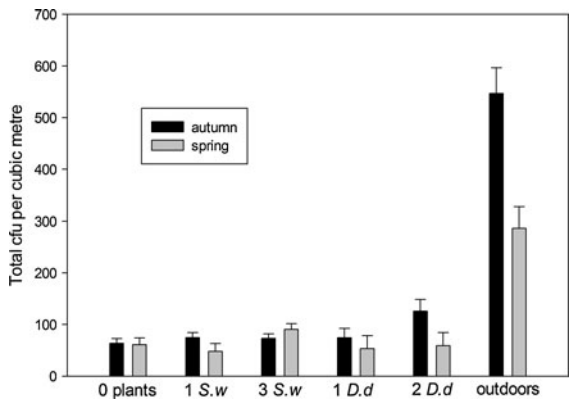

Fig. 1 Total cfu/m^3 for airborne fungal spore samples collected from outdoor air and offices with: no plants (0 plants), one *Spathiphyllum wallisii* (1 *S.w*), three *S. wallisii* (3 *S.w*), one *Dracaena deremensis* (1 *D.d*) and two *D. deremensis* (2 *D.d*) during the autumn and spring sampling periods (Mean ± SE; $n = 12$ for outdoor samples, $n = 33$ for indoor samples)

Table 1 Mean fungal cfu/m^3 (range) for genera identified in outdoor air samples and offices with: no plants (0 plants), one *S. wallisii* (1 *S.w*), three *S. wallisii* (3 *S.w*), one *D. deremensis* (1 *D.d*) and two *D. deremensis* (2 *D.d*) during the autumn sampling period

Genus	Autumn											
	0 plants		1 *S.w*		3 *S.w*		1 *D.d*		2 *D.d*		Outdoors	
	Mean fungal cfu/m^3 (range)		Mean fungal cfu/m^3 (range)		Mean fungal cfu/m^3 (range)		Mean fungal cfu/m^3 (range)		Mean fungal cfu/m^3 (range)		Mean fungal cfu/m^3 (range)	
Cladosporium	14.86	(3–29)	21.58	(0–59)	17.76	(4–28)	22.22	(0–57)	40.83	(0–155)	231.25	(0–450)
Alternaria	8.11	(0–24)	5.36	(0–11)	5.58	(0–15)	5.21	(0–17)	6.39	(0–13)	109.375	(50–250)
Penicillium	3.57	(0–13)	11.24	(0–50)	20.09	(0–38)	5.44	(0–10)	19.31	(0–66)	46.875	(0–250)
Rhizopus	2.98	(0–9)	1.49	(0–5)	2.46	(0–4)	2.78	(0–9)	1.77	(0–5)		
Nigrospora	2.28	(0–8)										
Pithomyces	1.34	(0–7)	2.98	(0–10)	0.82	(0–4)	2.31	(0–13)	5.38	(0–19)		
Scopulariopsis	1.34	(0–7)	2.53	(0–9)	2.53	(0–9)	3.47	(0–13)	1.56	(0–10)	3.125	(0–50)
Aureobasidium	1.29	(0–4)	4.02	(0–19)	0.35	(0–2)	1.39	(0–7)	0.52	(0–4)		
Malbranchea	0.82	(0–3)	1.12	(0–4)	1.64	(0–3)	0.46	(0–3)	2.40	(0–10)		
Scytalidium	0.82	(0–3)	1.56	(0–9)	2.06	(0–6)	13.31	(0–37)	2.19	(0–8)	3.125	(0–50)
Bipolaris	0.69	(0–4)	0.30	(0–2)								
Fusarium	0.69	(0–4)	0.30	(0–2)					1.77	(0–8)		
Ulocladium	0.69	(0–4)									6.25	(0–50)
Acremonium	0.60	(0–4)	0.30	(0–2)	0.60	(0–4)	2.55	(0–13)	1.25	(0–8)	31.25	(0–200)
Epicoccum	0.30	(0–2)	1.79	(0–4)	3.47	(0–9)	2.43	(0–7)	1.46	(0–4)		
Septate	0.30	(0–2)							0.69	(0–5)		
Aspergillus					0.99	(0–4)	0.23	(0–2)	3.09	(0–10)	15.625	(0–200)
Chaetomium									0.69	(0–4)		
Cladophialophora					1.04	(0–6)	1.04	(0–7)			6.25	(0–100)
Curvularia					1.39	(0–8)						
Geotrichum			1.04	(0–6)			1.04	(0–7)				
Gymnascella					1.04	(0–6)					37.5	(0–250)
Mucor			0.52	(0–3)							3.125	(0–50)
Oidiodendron							1.04	(0–7)				
Trichosporium							0.35	(0–2)				
Wallemia			0.87	(0–32)	0.30	(0–2)			1.50	(0–9)	6.25	(0–50)
Yeasts	14.60	(1–61)	11.20	(0–32)	4.78	(0–10)	4.74	(0–13)	5.20	(0–13)	29.5	(0–78)
Sterile mycelia	6.20	(0–22)	6.56	(0–12)	6.04	(0–36)	5.60	(0–25)	30.11	(0–122)	1.75	(0–50)

Indoor to outdoor (I/O) ratios amongst treatments and seasons are shown in Fig. 2. Although there was substantial variation in I/O ratios amongst treatment groups, these differences were not statistically significant ($P = 0.114$). No differences in I/O ratios between seasons were observed ($P = 0.143$). It is also clear that indoor numbers were far lower than outdoor counts.

We compared the diversity and relative abundance of fungal genera between seasons and treatments using ANOSIM. Unidentifiable colonies were not included in this analysis as they did not contribute reliable data to the species diversity amongst experimental groups. There was no significant difference in fungal community structure amongst seasons (Global R = 0.0152, $P = 0.418$). The absence of a seasonal pattern is surprising, especially considering that several abundant taxa showed seasonal differences when analysed independently (analyses not shown). However, there was a difference amongst treatments groups

Table 2 Mean fungal cfu/m^3 (range) for genera identified in outdoor air samples and offices with: no plants (0 plants), one *S. wallisii* (1 *S.w*), three *S. wallisii* (3 *S.w*), one *D. deremensis* (1 *D.d*) and two *D. deremensis* (2 *D.d*) during the spring sampling period

Genus	Spring											
	0 plants		1 *S.w*		3 *S.w*		1 *D.d*		2 *D.d*		Outdoors	
	Mean fungal cfu/m^3 (range)		Mean fungal cfu/m^3 (range)		Mean fungal cfu/m^3 (range)		Mean fungal cfu/m^3 (range)		Mean fungal cfu/m^3 (range)		Mean fungal cfu/m^3 (range)	
Cladosporium	13.13	(4–27)	8.42	(0–27)	21.09	(3–28)	11.27	(6–17)	23.35	(9–38)	81.82	(0–250)
Alternaria	9.60	(0–24)	5.38	(0–18)	10.53	(0–32)	6.77	(0–25)	7.04	(0–22)		
Epicoccum	6.14	(0–25)	6.42	(0–29)	10.60	(0–52)	4.46	(0–22)	6.71	(0–27)	4.55	(0–100)
Penicillium	5.13	(0–16)	5.21	(0–11)	14.03	(0–29)	2.64	(0–7)	6.94	(0–23)	15.91	(0–100)
Malbranchea	3.53	(3–6)	2.17	(0–9)	0.52	(0–4)	1.56	(0–7)	0.35	(0–2)		
Aspergillus	3.42	(0–19)	0.52	(0–2)	4.99	(0–16)	0.78	(0–4)	1.65	(0–4)	11.36	(0–50)
Pithomyces	2.75	(0–7)	2.52	(0–7)	1.41	(0–6)	2.46	(0–7)	0.52	(0–4)		
Wallemia	1.71	(0–4)	2.86	(0–10)	4.02	(0–15)	1.07	(0–5)	1.56	(0–7)	20.45	(0–200)
Nigrospora	1.49	(0–9)										
Scytalidium	1.45	(0–8)	3.13	(0–13)	2.60	(0–7)	3.13	(0–16)	2.72	(0–7)	2.27	(0–50)
Rhizopus	1.15	(0–4)	1.56	(0–4)	1.93	(0–6)	1.38	(0–5)	0.91	(0–2)		
Aureobasidium	1.08	(0–5)			0.78	(0–4)	1.34	(0–7)	0.52	(0–4)		
Curvularia	0.60	(0–2)					0.60	(0–4)			2.27	(0–50)
Gymnascella	0.52	(0–4)	0.00		2.60	(0–10)					11.36	(0–100)
Scopulariopsis	0.30	(0–2)	0.95	(0–4)	4.80	(0–18)	0.30	(0–2)	9.72	(0–59)	9.09	(0–50)
Cladophialophora	0.26	(0–2)	0.95	(0–5)	0.78	(0–4)	3.94	(0–22)	0.78	(0–5)	9.09	(0–200)
Acremonium					1.86	(0–7)	1.38	(0–4)	0.87	(0–4)	50.00	(0–300)
Arthrobotrys							0.52	(0–4)			4.55	(0–50)
Botrytis					0.52	0–4	0.52	(0–4)				
Chaetomium							1.04	(0–4)	0.26	(0–2)		
Fusarium							0.26	(0–2)				
Geotrichum											2.27	(0–50)
Mucor					0.26	0–2					2.27	(0–50)
Ulocladium											11.36	(0–100)
Yeasts			0.64	0–13	5.87	0–13	5.95	(0–13)	6.85	(0–14)	32.19	(0–200)
Sterile mycelia			7.03	0–13	7.22	0–13	8.04	(0–22)	20.35	(4–75)	6.51	(0–100)

(Global R = 0.399, $P = 0.01$). The pairwise differences between treatments showed a consistent pattern: All indoor samples had significantly different fungal community structure to the outdoor samples, whilst indoor groups were statistically similar.

To identify the taxa that contributed the greatest proportion of the differences between treatment groups, we used a SIMPER analysis. In all cases, *Cladosporium* spp. was the primary taxon differentiating amongst the treatments, contributing to 29.87–30.90 % of the overall between-group variance (average abundance in indoor samples = 16.3 cfu/m^3; in outdoor samples = 152.8 cfu/m^3). The other taxa that contributed more than 10 % of the between-group differences were *Acremonium* spp. (indoor = 0.91 cfu/m^3; outdoor = 44.4 cfu/m^3); *Alternaria* spp. (indoor = 7.07 cfu/m^3; outdoor = 48.61 cfu/m^3) and yeasts (indoor = 6.55 cfu/m^3; outdoor = 40.28 cfu/m^3).

Canonical correspondence analysis was performed on the indoor spore data to determine whether there were any multivariate relationships between the differences in mould diversity amongst indoor experimental groups and RH, temperature and outdoor

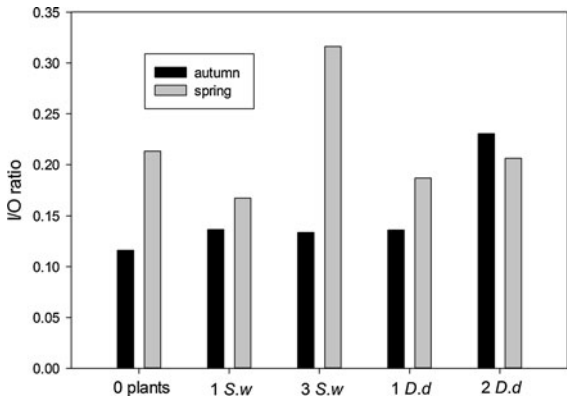

Fig. 2 Indoor/outdoor ratios for airborne fungal spore samples for offices with: no plants (0 plants), one *Spathiphyllum wallisii* (1 *S.w*), three *S. wallisii* (3 *S.w*), one *Dracaena deremensis* (1 *D.d*) and two *D. deremensis* (2 *D.d*) during the autumn and spring samples

spore counts and diversity. A very strong pattern was observed (biplot not shown), but all clusters of samples were associated with the date on which the samples were taken, rather than the tested experimental treatments (i.e. seasons and indoor plant treatment). Thus, short-term temporal variability subsumed any overall affects specific to temperature, RH or outdoor spore numbers.

There were no differences in either the quantitative or the qualitative aspects of the fungal communities associated with the two species of indoor plant.

4 Discussion

Despite the HVAC systems in both buildings, all indoor plant treatments increased indoor RH, which has been demonstrated previously (Costa and James 1999). Past work has detected variable relationships between RH and fungal spore counts. RH was positively correlated with airborne *Penicillium* spp. and *Aspergillus* spp. spore counts in O'Gorman and Fuller (2008). Conversely, Jantunen et al. (1987) found significantly decreased airborne fungal spore levels when indoor air RH was increased from 18–40 % to 38–62 %. There was no statistical correlation between RH and spore counts in the current study. This suggests that the quantitative increase detected was too small to have any effect on fungal behaviour, probably because the RH did not exceed 80 % throughout the study.

The mean indoor airborne spore counts detected in this study (48-126 cfu/m^3) were well below the World Health Organisation guidelines for the maximum indoor spore concentrations of plant and soil fungi of 500 cfu/m^3 (WHO 1990). Based on the guidelines proposed by Singh et al. (2010), the buildings had between very low (<50 cfu/m^3) and low (<200 cfu/m^3) spore concentrations. Additionally, the buildings had no visible mould on internal surfaces; no airborne pathogenic species (e.g. *Aspergillus fumigatus*) were found in high concentrations; the I/O ratios were less than 0.33, and our indoor samples had a species composition with a variety of both indoor and outdoor taxa, including common plant- and soil-associated genera such as *Cladosporium* and *Alternaria*. The indoor samples thus presented no aberrant health risk factors associated with the indoor aeromycota.

The presence of plants in an office did not significantly increase the number of fungal spores in the air. Whilst the pattern shown in Fig. 1 suggests a minor difference between heavily planted and unplanted offices, the variability amongst samples was of sufficient magnitude to subsume any inter-treatment differences. As the offices sampled were reasonably homogeneous with regard to their level of use and contents, this level of variability could be reasonably expected to be found in other functionally similar buildings as has found to be the case previously (Parat et al. 1997).

Whilst it could be predicted that indoor plants could act as a significant source of fungal material—any moist, organic material will harbour fungi; the critical question tested here was whether indoor plants contributed significantly to the gross spore load, or contributed large numbers of spores from pathogenic taxa to the indoor environment. Staib et al. (1978) detected the pathogens *Aspergillus fumigatus* and *Aspergillus niger* in the soil of ornamental plants, but only after not watering the plants for 2 weeks, so that the soil was dry, conditions that are known to strongly favour spore release from some fungal taxa (Pasanen et al. 1991). In our study, the soil was kept moist, in line with good indoor plant maintenance practice for the species used, so that spores would not have been easily released from the soil surface. Kenyon et al. (1984) detected pathogenic *Aspergillus* spp. in 9 out of 12 commercial potting mixes tested, but no realistic or systematic test was made of how and in what quantity these spores are released into indoor airspaces from

potted plants. Similar findings were reported by Summerbell et al. (1989). Reponen et al. (1992) found that indoor plants could contribute to increased indoor spore numbers, but only when the plants were disturbed or agitated. In our work, samples were taken when the plants were in an undisturbed state: All maintenance (watering and trimming of dead leaves) was performed after air samples were collected. It is thus possible that we could have detected greater spore loads if we had incorporated this treatment.

Although many fungal species can act as opportunist pathogens in immunocompromised hosts, some taxa have received more interest because of the increasing incidence of the diseases they cause. Of these, *Aspergillus* spp., especially *A. fumigatus*, are of primary concern as they can cause a range of saprophytic, invasive and allergic diseases, some with high mortality (O'Gorman 2011). Indoor plants have been implicated as a potential source of aspergilli (Engelhart et al. 2009; Hedayati et al. 2004; Lass-Flörl et al. 2000; Staib et al. 1978; Summerbell et al. 1989). We found that *Aspergillus* spp. indoors or outdoors, accounted for less than 5 % of the mould spores detected. We found only 1 occurrence of *A. fumigatus*, from a total of over 15,000 fungal colonies identified. These findings suggest that within the constraints of our sampling procedures and environmental conditions, the indoor plant species used in this study are not likely to be a major source of this species.

The dominant identifiable mould genera detected in the current study were *Cladosporium*, *Penicillium* and *Alternaria*. These taxa accounted for almost 50 % of all total identifiable mycoflora. Most fungal spore surveys are dominated by these genera, sometimes along with *Alternaria*, *Epicoccum* and *Aureobasidium* (Hargreaves et al. 2003). In previous Australian studies, Garrett et al. (1997) detected the same dominant genera in her samples from indoor and outdoor air in an urban area, whilst *Cladosporium* accounted for over 50 % of Hargreaves et al.'s (2003) samples from urban Brisbane. In a study on the effects of weather on outdoor airborne mould spore loads in Poland (Grinn-Gofron and Rapiejko 2009), the four most common genera found in spring and autumn were *Cladosporium, Ganoderma, Alternaria* and *Epicoccum*. In contrast to this, a study in Brazil (Gonçalves et al. 2010) found *Penicillium* and *Aspergillus* species to be dominant across all seasons, both indoors and outdoors.

Multivariate analysis between treatments revealed that the community structures of the aeromycota amongst indoor treatment group offices were statistically similar, and thus, the presence of plants within offices had no effect on their airborne fungal assemblages. No major community structure differences were found between indoor and outdoor samples. We detected a greater number of genera in indoor samples, but this effect was clearly due to the greater number of indoor samples collected. The overwhelming difference between indoor and outdoor samples was quantitative, with a similar array of taxa present in far larger numbers in outdoor air samples. This finding, taken together with the observation that there were no statistically significant differences in airborne spore loads or types between planted and unplanted offices, indicates that the indoor plants in the offices studied were not a significant source of indoor airborne mould spore loads.

Significant differences in mould spore density were found between indoor and outdoor air, with mould spore concentrations in the outdoor air being approximately an order of magnitude higher than in indoor air. The low indoor/outdoor ratios detected (0.11-0.32) were expected, since both building HVAC systems incorporate substantial filter matrices to remove particulates from the incoming air-stream, an especially important task as the buildings are located in the centre of a large city. Li and Kendrick (1995) found a similar pattern and concluded that HVAC systems reduced concentrations of airborne fungi. Similarly, Burge et al. (2000) detected higher numbers of spores in outdoor samples. In contrast, Mentese et al. (2009) found similar numbers of spores in indoor and outdoor samples, with the highest counts in high-humidity indoor spaces such as kitchens and bathrooms, possibly due to the inclusion of a broad variety of both air conditioned and naturally ventilated buildings, other than offices, in their samples.

Average total fungal spore numbers indoors across all treatments were 82 ± 13 cfu/m^3 during autumn and 62 ± 18 cfu/m^3 in spring. These concentrations were much lower than those found in other studies conducted in Australia, with Melbourne homes having 549 cfu/m^3 over a yearly period (Dharmage et al. 1999), homes in La Trobe Valley having a yearly median of 812 cfu/m^3 (Garrett et al. 1997) and homes in Brisbane having 810 ± 389 cfu/m^3 (Hargreaves et al. 2003). The latter two studies concluded that

proximity to parks or rural areas may increase levels of both indoor and outdoor viable fungal spores, which may have led to the higher spore numbers in these studies relative to the current work. In these studies, the buildings analysed were residential and were not equipped with central HVAC systems that the large office buildings in the current study possessed. One study of buildings with HVACs, conducted in the Perth (Western Australia) CBD, found an average of 264 cfu/m^3 with a minimum sample of 70 cfu/m^3 (Kemp et al. 2003) and concluded that the HVAC system did not reduce airborne fungal spore numbers. Contrary to our findings, the authors concluded that the HVAC system contributed to higher indoor concentrations of pathogenic fungi including *A. niger* and *A. fumigatus.* The discrepancy between Kemp et al. (1993) and our study may result from the variability in efficiency and maintenance between different HVAC systems.

Multivariate analyses were used in the current work to determine whether there were major differences between indoor and outdoor airborne fungal spore communities and thus provide evidence on whether outdoor air was the likely source of the indoor spores. The clear pattern emerging from these analyses was that it was varying proportions of a subset of fungal genera, rather than the presence or absence of specific taxa, which differentiated indoor and outdoor samples. In particular, the common mould genera: *Cladosporium, Acremonium* and *Alternaria* were more abundant in outdoor samples. This is evidence that the primary source of indoor moulds in the current work was from outdoors. Similarly, Lehtonen et al. (1993) showed that indoor fungal spores may be sourced from outdoor air and that the species composition of spores found inside parallel that of outside air. In contrast, Li and Kendrick (1995) found that indoor *Aspergillus* and *Penicillium* spores showed no seasonal patterns that correlated with outdoor phenology in these taxa and thus concluded that they have indoor sources.

The relationship between the indoor and outdoor airborne aeromycota is not completely understood, especially within sealed buildings with central HVAC systems. The results from this study, however, highlight the potential efficiency and effectiveness of modern HVAC systems to filter fungal loads from incoming air sources, an important adjunct finding from our work.

We did not detect seasonal variations in either the quantity or taxonomic diversity of airborne viable fungal spores. This contrasts with the findings of most previous research (e.g. Li and Kendrick 1995, Lehtonen et al. 1993) and was clearly a result of only sampling at two time points in our work. A detailed examination of seasonal mycoflora dynamics was not a major focus of our work: We sampled at different times simply to increase our sample size through time-for-space substitution. We thus make no conclusions about the phenology of fungi in our samples.

Use of the RCS air sampler potentially underestimates the total airborne mould spore density as the device can only detect fungal spores that are able to be cultured on the medium used and will not detect non-viable spores, unculturable spores or spores that have specific nutrient requirements (Flannigan 1997; Levetin 2004). However, the RCS sampler or functionally similar devices have been used extensively in previous research (e.g. Hargreaves et al. 2003; Takahashi 1997, Bonetta et al. 2010), making our data useful for comparison. This sampling method has been found to be more efficient at detecting certain fungal spores than the Anderson N6 sampler often used in air spora investigations (Saldanha et al. 2008), although the portable BioStage impactor has been found to produce higher culturable counts than the RCS (Zhen et al. 2009). The range of fungi we detected is similar to many previous studies that used Anderson samplers (e.g. Mentese et al. 2009; Green et al. 2003; Dharmage et al. 1999). Use of biochemical assays assessing ergosterol, mycotoxins or beta-glucans collected with the use of an impingement sampler have been shown to provide a more accurate assessment of total spore numbers (Miller and Young 1997); however, these do not allow for taxonomic identification of all the fungi present. It is clear that all methods of detecting airborne fungi have their own advantages and inefficiencies, and no two methods are directly comparable. We recognise that the method used in the current study has limitations and propose that firm conclusions on the effects of indoor plants to the fungal-associated aspects of indoor air quality should only be made after realistic field trials have been made with a variety of sampling procedures.

5 Conclusions

The current work represents the first systematic field study of the relationship between indoor plants and the

indoor air spora in air-conditioned buildings. We found no evidence of a relationship between indoor plants and neither the quantity nor taxonomic structure of the airborne fungal community. We acknowledge that the preclusion of any natural biological material from proximity to highly immunocompromised individuals is desired (especially for hospitals); however, in ordinary workplace environments such as those analysed in the current experiment, we can find no evidence supporting any potential health risks related to indoor plants and fungal spore emissions. Whilst our findings provide evidence that indoor plants do not make a significant quantitative contribution to the indoor aeromycota, further research is required to determine the effect of indoor plant maintenance regimes, other indoor plant species and growth substrates on spore release into indoor air.

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