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Seasonal distribution of pollen in the atmosphere of Darwin, tropical Australia: Preliminary results

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Abstract
Pollen loads in the atmosphere of Darwin, a city located in the wet-dry tropics of Australia, have been monitored for the period March 2004 to November 2005 as part of a large research program looking at atmospheric particles and human health. Seven pollen types dominate the pollen spectrum, the herbaceous families of Poaceae (grasses) and Cyperaceae (sedges), as well as several native tree and shrub taxa, *Acacia*, *Callitris*, *Casuarina*, Arecaceae and Myrtaceae. The pollen loads were found to have a strong seasonal component associated with the alternating wet (November to March) and dry (April to October) seasons of the region. Seventy percent of the yearly pollen load is captured during the dry season, with the peak pollen period occurring at the onset of the dry season (April–May) when most grasses are in flower. The daily pollen concentration decreases as the dry season progresses, accompanied by a change in composition; fewer herbaceous but increasing woody taxa. Preliminary health outcomes reveal a positive association between hay fever, Poaceae and Acacia pollen, as well as a significant association between total fungal spore concentrations and asthma. The Darwin record contrasts significantly with surveys conducted in the subtropical and temperate cities of Australia where temperature as opposed to rainfall and the prevalence of northern hemisphere exotic tree species have a greater influence over the seasonality and composition of the pollen loads.

Keywords: *Atmospheric pollen, Darwin, wet-dry tropics, pollen season*

The first aerobiology study for the wet-dry tropics of Australia has been undertaken in Darwin as part of the Darwin Smoke Project. This multidisciplinary research program aims to identify the causes and consequences of high air pollution episodes in the Darwin region. In particular the program aims to determine the ecological causes and adverse health effects of different levels of bushfire smoke in Darwin with a preliminary study suggesting a link between smoke pollution levels and asthma (Bowman & Johnston, 2005).

Darwin's situation as a major population center is somewhat unique, as the only source of air pollution is the high incidence of controlled and uncontrolled bushfires during the dry season (Bowman & Johnston, 2005). Therefore disentangling the contributions that various aerosol particles make to respiratory health issues is more achievable here than in some other parts of the country.

The research program has a number of components including atmospheric chemistry, meteorology, landscape ecology, epidemiology and aerobiology. Here we present the results of the aerobiology study, a 20-month comparative study of the pollen and spore composition for two monitored sites.

Study area

Darwin, with a population of just over 100 000, is located in the wet-dry tropics of Northern Australia (Figure 1). The two sampling sites are located

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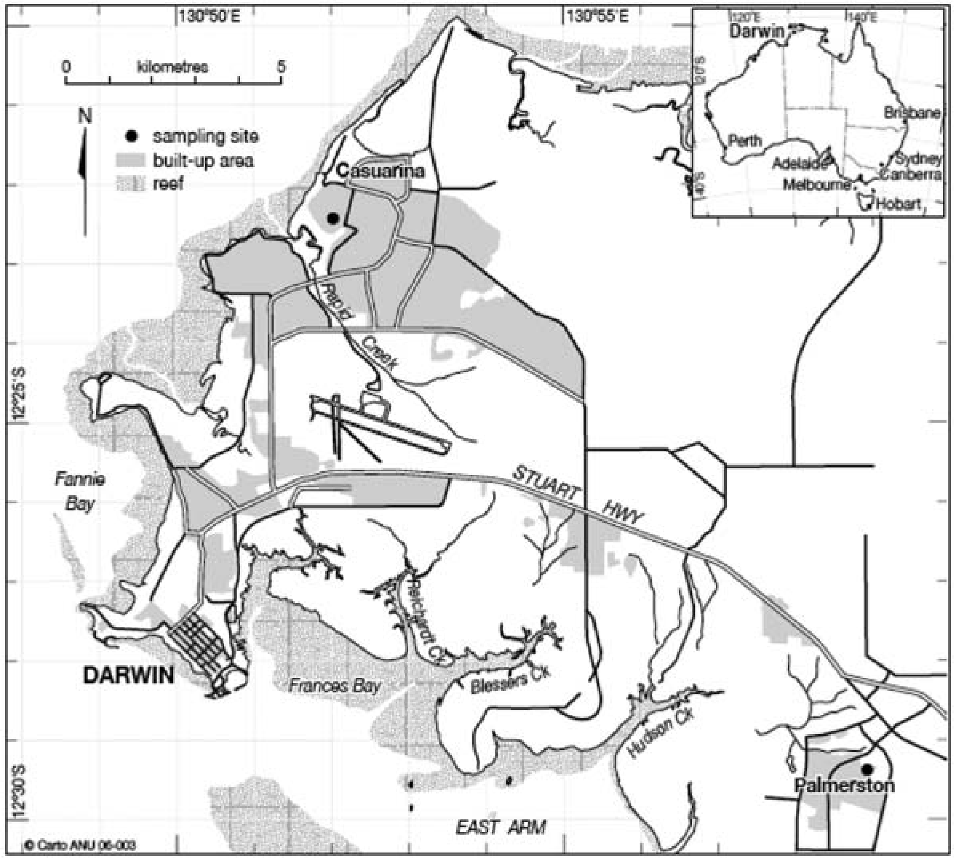


Figure 1. Location map of the Darwin region and the two monitoring sites, Casuarina and Palmerston.

20 km apart within the grounds of Charles Darwin University, one at the Casuarina Campus and one at the Palmerston Campus (Figure 1). The Casuarina Campus is located in a well-established northern suburb of Darwin where the vegetation is composed of tropical urban plantings dominated by palms.

The Palmerston Campus is located on the southern outskirts of Darwin in an area of relatively recent urban expansion, and is exposed to more of the natural vegetation in the Darwin region than the Casuarina Campus. Frequently burnt *Eucalyptus* savannas, with their associated understories of grasses and tropical shrubs and *Acacia* and grasses, dominate this landscape. There are also large plantations of the Australian conifer *Callitris intratropica*. Darwin has a distinct wet and dry season with little variation in temperature (Table I). The average annual maximum temperature is 32°C and the average yearly rainfall 1 714 mm. The wet season is centred round the Austral summer months (November to March), while the driest months occur over Austral winter (April to October).

Material and methods

Aerobiological data were collected using two seven-day Burkard volumetric samplers (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK) located on rooftops at Casuarina and Palmerston, each approximately 14 m above the ground. Both traps were calibrated to sample air at 10 l/minute and each sampling period ran for seven days. Airborne particles were initially trapped on an adhesive surface composed of Vaseline and 10% paraffin wax in toluene on Melinex™ tape (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK). Silicon based adhesive (Lanzoni s.r.l., Bologna, Italy) was used from November 17, 2004 to November 2005. The seven-day tapes were then cut into 24-hr segments and mounted on glass slides with fuchsine stained Gelvatol (Lacey & West, 2006). A comparative test of the two different adhesives has not been carried out.

Analysis of each 24 h period was conducted by counting four transects at 400X magnification. Pollen and spore counts were then converted to grains/m^3 of air and expressed as a daily mean value (Hirst, 1952). Calculation of the daily mean concentration of fungal spores was calculated in the same manner, although this was based on a single transect counted at 400X magnification and detailed fungal spore identification was not attempted. Pollen identification was aided by the

Table I. Mean annual meteorological data for Darwin Airport.

	Months											
	WET			DRY							WET	
Meteorological parameters	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean Daily Max Temp (deg C) (annual average 31.9)	31.8	31.4	31.9	32.7	32.0	30.6	30.5	31.3	32.5	33.1	33.2	32.5
Mean Daily Min Temp (deg C) (annual average 23.2)	24.8	24.7	24.5	24.0	22.1	20.0	19.3	20.5	23.1	25.0	25.3	25.3
Mean Rainfall (mm) (annual average 1 714)	423.3	361.1	319.3	98.9	26.5	2.0	1.4	5.7	15.4	70.7	141.8	247.9
Mean no. of Rain days (annual average 111)	21.1	20.3	19.3	9.1	2.3	0.6	0.5	0.6	2.3	6.6	12.1	16.5
Mean 3pm Relative Humidity (%)	70.0	72.0	67.0	53.0	43.0	38.0	38.0	41.0	48.0	53.0	59.0	65.0
Mean Daily Evaporation (mm)	6.5	5.9	6.1	6.8	7.1	7.0	7.2	7.5	8.0	8.3	7.8	7.0

existing reference collection held in the Department of Archaeology and Natural History at ANU and by a pollen reference collection compiled specifically for this project. This was carried out through the regular collection of flowering material around both sites. This pollen reference material will shortly be available as an online database that can be accessed at http://palaeoworks.anu.edu.au.

There were periodic breakdowns of the two samplers, with the Casuarina sampler proving to be the more reliable. The Palmerston record runs from April 1, 2004 until March 31, 2005, with 85 days of missing of data. The Casuarina record runs from March 18, 2004 until November 15, 2005, with only 58 days of missing data. The majority of the missing days are during the wet season due to equipment failure, possibly caused by the increased air humidity.

Results

There is a strong seasonal signal in the pollen composition at each site, with the daily pollen load highest during April at the onset of the dry season (Figure 2). Seven pollen types dominate the pollen loads at the two sites; Poaceae (grasses), Cyperaceae (sedges), Arecaceae (palms), Myrtaceae (*Eucalyptus*, *Melaleuca* etc), *Callitris* (cypress pine), *Casuarina* (she oak) and *Acacia* (wattles). These seven pollen types account for 90% of the pollen trapped, with

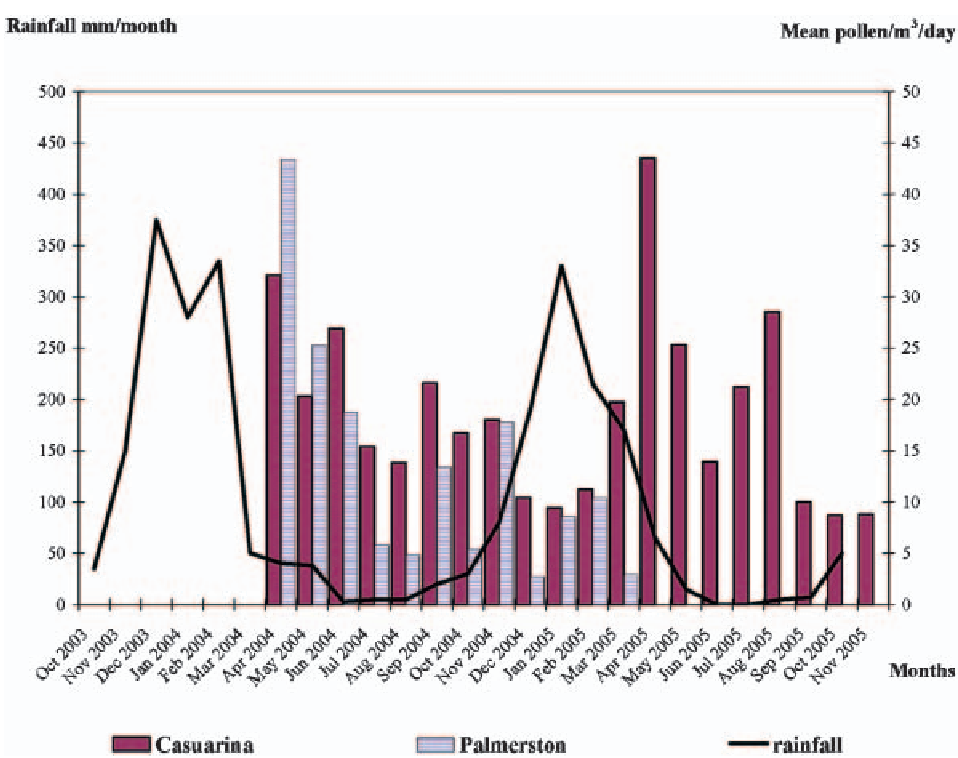


Figure 2. Average daily pollen concentrations (grains/m^3) per month for the two sites Casuarina and Palmerstone plotted with monthly rainfall (mm; recorded at Darwin Airport).

the remaining 10% composed of around 30 different pollen types seen only occasionally and in low numbers.

The peak pollen emission period is during the dry season, from April to November, with 70% of the yearly total captured during these months. However, within this season there is great monthly variation. In 2004 the average daily pollen concentrations for April–November ranged between 25.5 grains/m^3 to 11.2 grains/m^3 at Casuarina, and from 34.5 grains/m^3 to 4.2 grains/m^3 at Palmerston (Figure 2). For the same period in 2005 only the Casuarina sampler was operating, recording a range in average daily pollen concentrations of between 32 grains/m^3 and 7 grains/m^3.

Three of the main pollen taxa are most prevalent at the onset of the dry season, Poaceae, Cyperaceae and *Acacia* (Figure 3). However, as the dry season progresses, the pollen concentration of the atmosphere decreases, especially the representation of herbaceous taxa. In April 2004 the pollen loads at both Casuarina and Palmerston were dominated by a combination of grass and sedge pollen, 74% at Casuarina and 92% at Palmerston. However, in April 2005, the pollen loads sampled at Casuarina were dominated by Myrtaceae pollen (33%), with Poaceae and Cyperaceae making up just 42% of the pollen load, illustrating the year to year variation in pollen composition.

The two sites show broad similarities in the timing of peak pollen loads, although the composition of the pollen is different and related to the location of each sampling station. Palmerston on the outskirts of Darwin has a greater representation of pollen from the native woodlands surrounding Darwin, grasses, sedges, Myrtaceae and *Callitris*. At Casuarina Campus the pollen spectrum is dominated by grasses, palms and Myrtaceae.

While *Casuarina* pollen are intermittent and occur in low numbers in the Palmerston record, the concentrations of *Casuarina* pollen are more consistent at the Casuarina Campus (Figure 3). This record has the same peak emission periods, July and September, recorded for both dry seasons, but with the concentrations in September 2004 six times greater than those in 2005. *Callitris* pollen peaks in May and June 2004 at both sites, with concentrations for both samplers peaking again at the onset of the 2004/2005 wet season (Figure 3). The May/June peak in the 2004 record is not repeated in the 2005 record at Casuarina, and the wet season peak is only hinted at the tail end of the 2005 record.

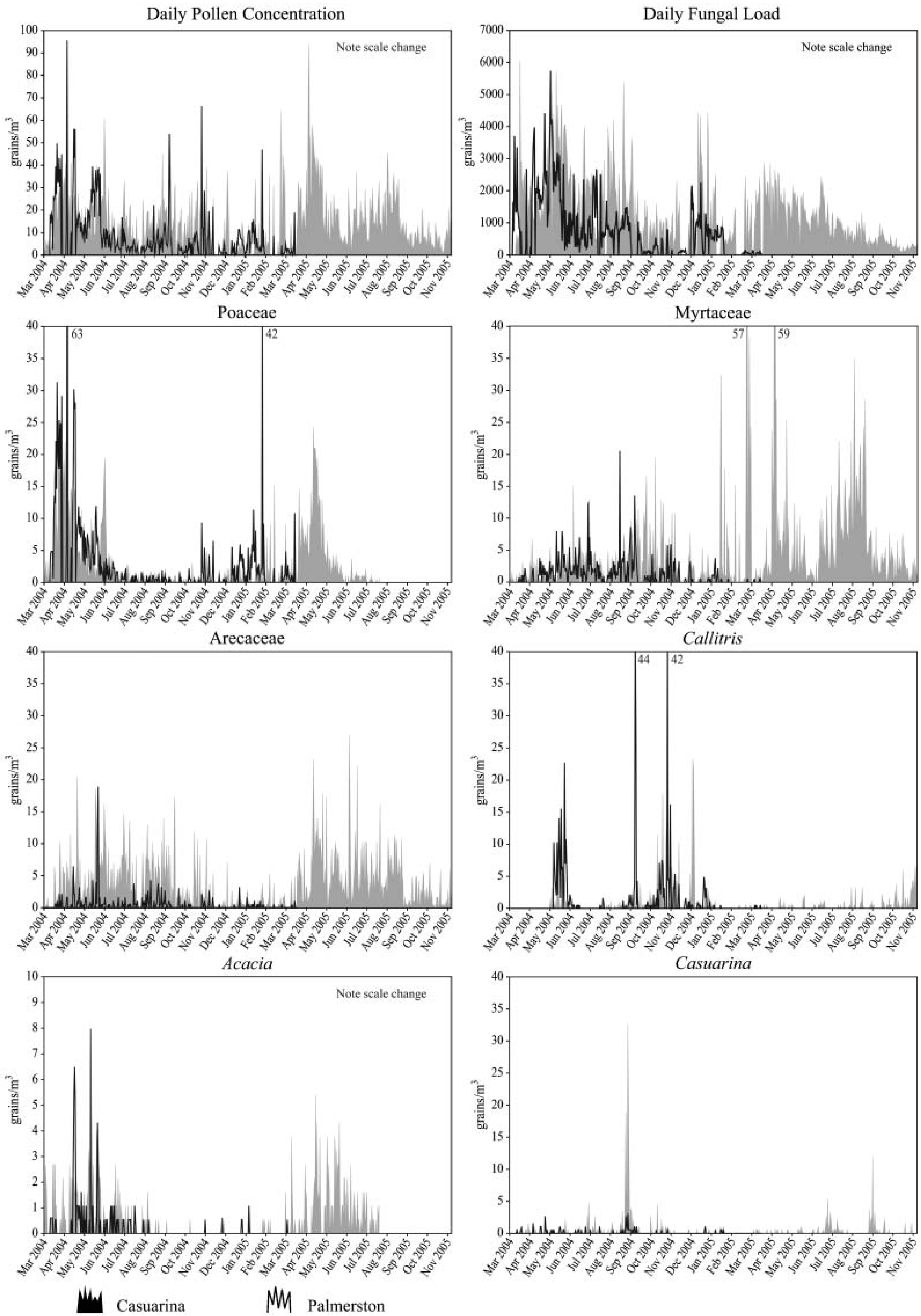
The Myrtaceae pollen concentrations are different between the sites and there is a distinct seasonal variation in the longer record from Casuarina. For the 2004 dry season average daily concentrations are similar at both sites, and the flowering of the eucalypt woodlands at the end of the dry season (Brock, 2001) is captured well at both stations (Figure 3). However, the peak emission periods at Casuarina for 2005 vary considerably from 2004 (Figure 3). Also, during the closing months of the 2004/2005 wet season, Myrtaceae pollen barely registers in the Palmerston record, yet in the Casuarina record, peak values are recorded that carry on into the early months of the dry season.

In general the total fungal spore load exhibits a similar seasonality to the pollen concentration data (Figure 3); although at this stage the fungal spores remain undifferentiated. The annual concentrations vary between the two sites, with Casuarina recording a total annual concentration of 512 160 spores for March 2004 to April 2005, while Palmerston recorded less than half that with 229 060 spores. In both cases the peak spore time was at the onset of the dry season, with atmospheric spore loads diminishing as the dry season progressed. There was also a short-lived peak at the start of the wet season in January and February. For both sites there were more spores in the atmosphere during the dry season than during the wet season. The two dry seasons recorded at Casuarina also vary considerably (Figure 3) with a total of 380 180 spores/m^3 for April to November 2004 and almost half that with only 208 200 spores/m^3 for April to November 2005.

Discussion

There is a strong regional signal in the pollen data, while at the same time there are subtle differences between the sites reflecting their different situations. The Casuarina sampler has a more urban location, while the Palmerston site is more peripheral and in closer proximity to the regional savanna landscape. This is reflected in the fact that the highest values of grasses, sedges, and *Callitris* occur at Palmerston, while the Casuarina campus has the highest values of Arecaceae (palms) and *Casuarina* pollen, reflecting the proximity of the sample location to landscape plantings and the coast. The domination of palms in the pollen spectrum at the Casuarina sampling site is possibly due to an extensive planting of *Roystonea regia* (Cuba Royal Palm) close to the pollen and spore sampler.

Daily pollen counts are highest during the dry season from April–November, capturing 70% of the yearly total, with the peak emission period occurring at beginning of dry season, April to May, when grass and sedge pollen dominate. The timing of peak pollen concentration in the grasses, wattles and



Daily Pollen Concentration
Note scale change
Daily Fungal Load
Note scale change
Poaceae
63
42
Myrtaceae
57
59
Arecaceae
Callitris
44
42
Acacia
Note scale change
Casuarina
grains/m3
Mar 2004
Apr 2004
May 2004
Jun 2004
Jul 2004
Aug 2004
Sep 2004
Oct 2004
Nov 2004
Dec 2004
Jan 2005
Feb 2005
Mar 2005
Apr 2005
May 2005
Jun 2005
Jul 2005
Aug 2005
Sep 2005
Oct 2005
Nov 2005
Casuarina
Palmerston

palms are the same for both years in the Casuarina record, as are the quantities, suggesting there may be a predictable pollen season for Darwin (Figure 3).

All of Australia's cities have a grass allergy season, although the peak emission period for grass pollen varies with climatic zone. For temperate Melbourne, with its winter rainfall regime, grass pollen peaks during spring and early summer, November to January (Ong et al., 1995*a*, *b*). For Sydney, which has a more even distribution of yearly rainfall, grass pollen is at a maximum from spring through to autumn, October to May (Bass et al., 1991; Bass & Morgan, 1997). In sub-tropical Brisbane the grass pollen season is from summer through to autumn (December to April) (Green et al., 2002), while in Darwin it is during the onset of the dry season (April to May).

Identifying grass pollen down even to genus level can be very difficult. Therefore it is not known what component of the grass pollen in the Darwin study is derived from native species and what is derived from introduced species. It is noted that for the northern tropics a number of the grasses introduced for livestock purposes are considered to be serious exotic weeds and that the most serious of these flower early in the dry season (Bowman, 2005). These grasses may therefore be posing health problems as well as ecological ones.

An important finding of the present study is that the grasses, wattles and palms, which are all known to be allergenic, (Chakraborty et al., 1996; Chowdhury et al., 1998; Boral, 2000; Chew et al., 2000; Singh & Kumar, 2003), have a potentially predictable flowering season. The peak pollen periods for *Callitris* and *Casuarina* may also prove, with further monitoring, to be predictable, although at present the variation in pollen concentration between years is considerable. The Myrtaceae are possibly the least predictable and show strong variation throughout the record. An important contributing factor is that it has been difficult to confidently differentiate the Myrtaceae pollen. Therefore the category represents a broad range of ecological habitat, from swamps and riparian zone vegetation to savanna woodland, and the influence of meteorological factors on each of these.

Green et al. (2004) looked specifically at the relationship between meteorological factors and the pollen seasons of three native arboreal taxa; namely Casuarinaceae, Cupressaceae and Myrtaceae. They separated out these three families from a five-year aeropalynology study for Brisbane and analysed the average monthly loads against meteorological data. They found that the major factors influencing the pollen loads were pre-seasonal precipitation and pre-seasonal maximum temperature. The current data set for Darwin is still too short for such an analysis, as it does not capture all the possible yearly variation in the regional pollen loads. However, ongoing monitoring and analyses will eventually address some of these issues in the Darwin data, which are ultimately essential for producing predictive models that can be applied to the management of respiratory health.

The three major cities on the east coast of Australia, Brisbane, Sydney and Melbourne, have had several pollen studies conducted to assess air quality and its links with respiratory allergy (Rees, 1964; Moss, 1965, 1967; Ong et al., 1995*b*; Bass & Morgan, 1997; Rutherford et al., 1997; Green et al., 2002). A comparison of the Darwin pollen loads with the loads from these three cities highlights some important differences (Table II), although, unfortunately, comprehensive pollen concentration data for Melbourne could not be found. The first obvious difference is that Darwin has the lowest annual pollen load. Sydney has much higher pollen loads than either Darwin or sub-tropical Brisbane, and presumably this would also be the case for Melbourne as well, as the pollen spectrum in both these cities is dominated by exotic tree taxa that have aerially dispersed pollen, such as birch, maple, olive and privet (Ong et al., 1995*a*, *b*; Bass et al. 1997). By contrast, in Brisbane and Darwin, pollen derived from woody taxa is dominated by native species, with their largely entomophilous pollination strategies.

It is the domination of the pollen spectrum by native species that makes the record somewhat unique in the Australian context, enabling health workers to gain a better understanding of the impact of taxa such as *Acacia*, which although having been implicated in allergenic disorders overseas (Ariano et al., 1991; Boral, 2000; Chew et al., 2000), has only been suspected as a cause of hayfever in Australia. *Acacia* pollen was most abundant in the Darwin atmosphere from March to September for our sampling period.

Many of the Cupressaceae are known to be aeroallergens, including some of the Australian natives (Bass et al., 1991; Pham et al., 1994). However, the native species cannot be distinguished from the exotic species using light microscopy. In Sydney, Cupressaceae pollen makes up 28% of the yearly pollen load, with most of this pollen type probably originating from urban plantings of introduced species. By contrast, in Brisbane, where Cupressaceae pollen is around 9% of the yearly pollen load, Green et al. (2004) considered there to

Figure 3. Daily variability in total pollen and fungal spore concentrations for Casuarina and Palmerston monitoring sites. Also shown is the daily concentration for Poaceae, Myrtaceae, Arecaceae, *Acacia*, *Callitris*, and *Casuarina*.

Table II. Average annual concentrations and relative percentages of the major pollen taxa in the Darwin study compared with the data from Brisbane, Sydney and Melbourne.

	Analyzed sites							
	Darwin		**Brisbane**		**SW Sydney**		**Melbourne**	
Taxa	Av. Ann. grains/m^3	%	Av. Ann. grains/m^3	%	Av. Ann. grains/ m^3	%	Av. Ann. grains/m^3	%
Poaceae	950	26.0	4 630	72.0	4 950	18.0	4 220	20.0
Cyperaceae	373	10.0	90	1.0	78	0.5	not reported	not reported
Arecaceae	638	17.0	–	–	–	–	not reported	not reported
Casuarinaceae	113	3.0	420	7.0	1 800	6.0	not reported	not reported
Cupressaceae	373	10.0	570	9.0	6 580	23.0	12 660 (Cupressaceae+*Ulmus*)	60.0
Acacia	100	2.5	10	0.1	90	0.5	not reported	not reported
Myrtaceae	772	21.0	206	3.0	3 145	11.0	not reported	not reported
Other	371	10.0	540	8.0	11 614	41.0	4 220	20.0
Av Ann Total	3 693		6 466		28 257		21 100	
Variation			4 600–8 500 grains/m^3 5yr record		12 500–41 500 grains/m^3 3yr record			

The Darwin values are one calendar year (April 2004 through to the end of March 2005) and are an average of the two sampling sites. The values for the other cities are the yearly averages of multiyear studies (Green et al., 2002; Bass & Morgan, 1997; Ong et al., 1995*a*, *b*). NB: Melbourne Data – the only yearly values reported by Ong et al. (1995*a*, *b*) are for grass and Cupressaceae+*Ulmus*. All other values have been inferred.

be a much greater input from native *Callitris* species. In Darwin *Callitris intratropica* is the dominant Cupressaceae species in the landscape, with *Callitris* pollen comprising round 11% of the yearly pollen load (Table II). *Callitris intratropica* is known to fruit between June and October (Brock, 2001) making the second peak in November a curiosity. Refloating of pollen or long distant transport by inland winds is a possibility (e.g., Green et al., 2004; Stennett & Beggs, 2004) and will be tested with ongoing monitoring and statistical analyses that incorporate meteorological data.

A number of Australian taxa have been introduced to other countries, and several studies have shown that *Eucalyptus* and *Melaleuca* species, as well as *Casuarina equisitifolia* can cause allergic responses (Green et al., 2004). The dominance of pollen loads by native woody taxa in the latter part of the dry season will potentially provide the first good test of whether these taxa contribute in any significant to way to hayfever incidence in Australia. F. H. Johnston is examining daily pharmacy sales treatments for hay fever, including selected antihistamines, decongestants and nasal steroids. An interim analysis using data from the first year of pollen and spore monitoring found significant associations between pharmacy product sales and Poaceae and *Acacia* pollen (F. H. Johnston, pers. comm., 2006). Analysis of the full 2 years of data is currently in progress.

Although there are only a few published studies of volumetric fungal spore sampling for the tropics, on the whole the fungal load data from Darwin is in keeping with that from other tropical regions (Lim et al., 1998). In Bangkok, the peak fungal spore periods are during the driest months, December and January (Phanichyakarn et al., 1989 cited in Lim et al., 1998), with the lowest values at the start and end of the rainy season. While Singapore is considered to have two distinct monsoon seasons, unlike Darwin, it is not considered to have a distinct wet or dry season. However, rainfall maximum tend to occur in December and April and the drier months are usually February and July (http://app.nea.gov.sg/). In a five-year study by Lim et al. (1998) the major peaks in the fungal spore load were found to occur in February and March and again in October and November. A minor peak also occurred in July, one of the drier months. The mean daily spore concentration of 1 688 spores m^{-3} day^{-1} is comparable with the data from Darwin, which has a mean daily concentration of 1 464 spores m^{-3} day^{-1}. However the daily maximums vary considerably, with 19 075 spores m^{-3} day^{-1} recorded for Singapore and around 6 050 spores m^{-3} day^{-1} for Darwin.

Analysing the daily concentrations of individual fungal taxa within the Singapore data against an array of climatic variables resulted in a range of differing correlations, illustrating that the presence and movement of fungal spores around environment is quite complex. As yet detailed analyses of the spore taxa from Darwin have not been attempted.

The presented data are also being used in two studies of asthma. In the first we followed the daily asthma symptoms and medication use in a panel of 250 people with doctor diagnosed asthma from April to November 2004. After adjusting for potential confounders including temperature, humidity, particulate air pollution and influenza, no association with any health outcome was found with either the total pollen count or the counts for individual taxa. However, significant associations between the total fungal spore count and both onset of asthma symptoms and commencement of reliever medication were observed. There was an approximate 10% increase in the proportion of participants who became symptomatic with their asthma (OR 1.10 95%CI 1.02–1.20, $p=0.011$) and 20% rise in the proportion commencing reliever medication (OR 1.20 95%CI 1.014–1.70 $p=0.020$) with each rise of 1 000 in the fungal spore concentration (Johnston et al., in press). These results are consistent with previous studies of asthma exacerbations in relation to fungal spore concentration (Atkinson et al., 2006; Delfino et al., 1997). The second study is also examining the association between pollen and spore concentration and daily counts of emergency department attendances and admissions to hospital for asthma. Data analysis for this is currently in progress.

Conclusions

This study has produced the first continuously measured pollen data for Darwin and tropical Australia. It has demonstrated that 70% of the yearly pollen load occurs during the dry season, with the peak loads of common allergy causing pollen occurring in the first two months of the dry season. The study has also demonstrated that these taxa, such as the grasses, have predictable seasonal occurrences, a finding that is important for respiratory health management in the area. Most of the native tree taxa appear to have predictable peak pollen periods; however the actual loads can very considerably from one year to the other. The Myrtaceae family proved to be the least predictable, but this large umbrella category has captured an array of habitat types that respond in different ways to changing meteorological conditions. Continued monitoring will provide a more substantial dataset suitable for statistical analyses focused untangling the processes behind seasonal variation in Darwin's pollen signature. This future work will ultimately lead to a predictive model of pollen loads for the Darwin region, an important tool for managing respiratory health.

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