

Aeropalynology of Australian native arboreal species in Brisbane, Australia

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Abstract

The influence of meteorological parameters on airborne pollen of Australian native arboreal species was investigated in the sub-tropical city of Brisbane, Australia over the five-year period, June 1994–May 1999. Australian native arboreal pollen (ANAP), shed by taxa belonging to the families Cupressaceae, Casuarinaceae and Myrtaceae accounts for 18.4% of the total annual pollen count and is distributed in the atmosphere during the entire year with maximum loads restricted to the months May through November. Daily counts within the range 11–100 grains m⁻³ occurred over short intervals each year and were recorded on 100 days during the five-year sampling period. Total seasonal ANAP concentrations varied each year, with highest annual values measured for the family Cupressaceae, for which greater seasonal frequencies were shown to be related to pre-seasonal precipitation ($r^2 = 0.76$, $p = 0.05$). Seasonal start dates were near consistent for the Cupressaceae and Casuarinaceae. Myrtaceae start dates were variable and established to be directly related to lower average pre-seasonal maximum temperature ($r^2 = 0.78$, $p = 0.04$). Associations between daily ANAP loads and weather parameters showed that densities of airborne Cupressaceae and Casuarinaceae pollen were negatively correlated with maximum temperature ($p < 0.0001$), minimum temperature ($p < 0.0001$) and precipitation ($p < 0.05$), whereas associations with daily Myrtaceae pollen counts were not statistically significant. This is the first study to be conducted in Australia that has assessed the relationships between weather parameters and the airborne distribution of pollen emitted by Australian native arboreal species. Pollen shed by Australian native Cupressaceae, Casuarinaceae and Myrtaceae species are considered to be important aeroallergens overseas, however their significance as a sensitising source in Australia remains unclear and requires further investigation.

Abbreviations: ANAP – Australian Native Arboreal Pollen

1. Introduction

Aeropalynological studies conducted in Australia have previously focused on the temporal distribution of the major components of the airborne pollen load, including the allergenically important family, Poaceae (Bass and Morgan, 1997; Ong et al., 1995; Smart et al., 1979). Although the allergenic importance of

Australian native arboreal pollen (ANAP) has been documented (Bass et al., 1991; Garcia et al., 1997; Hunter et al., 1996), the impact of climatic variables on airborne pollen loads of these taxa remains unclear and has not been quantitatively analysed previously in Australia. Australian native species belonging to the families Cupressaceae, Casuarinaceae, and Myrtaceae form extensive sclerophyllous, riparian and wetland

communities and their pollen, which is disseminated by a range of animal, insect and anemophilous vectors are a significant component of airborne loads in the atmosphere of Brisbane (Green et al., 2002).

The allergenic significance of pollen belonging to the family Cupressaceae is well recognized throughout the Northern Hemisphere. This family produces spheroidal, intectate anemophilous pollen grains that are small in size and are often confused with pollen from the Taxaceae and Taxodiaceae (Figure 1). Cupressaceae pollen has commonly been reported as an important aeroallergen and causal factor of autumn and winter pollinosis in Europe and Japan (Guerra et al., 1996; Mari et al., 1996; Yasuda et al., 1986). The importance of allergy to Cupressaceae pollen in Australia has only recently been established (Bass et al., 1991; Pham et al., 1994). Environmental atmospheric surveys have detected airborne Cupressaceae pollen at varying concentrations and intervals during winter and spring in several major Australian cities (Bass et al., 2000). In Brisbane, Cupressaceae pollen accounts for 8.7% of the total yearly atmospheric pollen count (Green et al., 2002; Moss, 1965), with up to 5.8% of allergic individuals in the region sensitised to pollen allergens belonging to this family (Wright and Derrick, 1975).

More recently, attention has focussed on the clinical implications of exposure to pollen belonging to the Australian native families Casuarinaceae and Myrtaceae (Figure 1). Australian native species, including *Melaleuca quinquenervia*, *Melaleuca leucadendron*, *Callistemon citrinus*, *Casuarina equisetifolia* and *Eucalyptus* spp. have been shown to induce respiratory symptoms and allergic sensitisation in individuals located in North America (Bucholtz et al., 1987; Stablein et al., 2002), Spain (Garcia et al., 1997), Malaysia (ChoonKook et al., 1998), Taiwan (Wang and Chen, 1992), Thailand (Dhorranintra et al., 1991), and India (Prakashkumar et al., 1998). However, in Australia few studies have been undertaken assessing the clinical, biochemical and aerobiological relevance of Casuarinaceae and Myrtaceae pollen.

The effect of climatological parameters on the airborne loads and temporal distribution of ANAP has remained relatively unclear in Australia, although it is well known from European studies that pollen belonging to indigenous taxa of the Cupressaceae is affected by weather parameters particularly temperature and hours of sunshine (Ariano et al., 1994). This is the first study to assess the role of meteorological parameters on the timing and incidence of ANAP in

a sub-tropical Southern Hemisphere environment and follows our preliminary investigation of the airborne pollen content of Brisbane (Green et al., 2002). To further understand the aerobiologic significance of Australian native pollen types we aimed to quantitatively and statistically analyse the ANAP seasons based on five-years (June 1994–May 1999) of environmental pollen monitoring.

2. Materials and methods

Airborne ANAP represented by the Cupressaceae, Casuarinaceae and Myrtaceae was collected over a five-year period (June 1994–May 1999) in the sub-tropical city of Brisbane (27°29' S 153°8' E), south-eastern Queensland, Australia. The trapping site was located in the suburb of Rocklea, 7 km south-east of the central business district on a Queensland Department of Primary Industries research farm. Elevation of the site is 12.5 meters above sea level. Brisbane experiences a sub-tropical climate (average yearly mean temperature 25.9°C) with a summer rainfall regime (average yearly rainfall 1114.6 mm) (Bureau of Meteorology, 1983). The major vegetation formation of the trapping site is that of open *Eucalypt* woodland with an understorey of Poaceae and herbaceous species and where surrounding creeks and rivers are vegetated by riparian communities as previously described (Green et al., 2002). The Myrtaceae is represented at the trapping site by *Eucalyptus crebra*, *E. tereticornis*, *Corymbia citriodora* and *Melaleuca linarifolia*; the Casuarinaceae by *Casuarina cunninghamii* and the Cupressaceae by *Callitris* spp. Additionally, gardens of surrounding residential and commercial areas have numerous native representatives of the Myrtaceae and Casuarinaceae and both native and exotic taxa of Cupressaceae.

Aerobiological data was collected using a seven-day Burkard volumetric spore and pollen trap (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK), which was elevated two meters above ground level and operated near continuously at the Rocklea site between June 1994 and May 1999. Several periods of motorized trap breakdown were experienced, but these were usually brief and accounted for a total of 117 days during the entire sampling period. The trap was calibrated to continuously sample air at ten litres per minute and the atmospheric particulate matter was deposited onto tapes coated with a thin film of Dow Corning pressure-



Figure 1. Photomicrographs of pollen belonging to Australian native arboreal families (left to right). A, Cupressaceae. B, Casuarinaceae. C, Myrtaceae.

sensitive silicone adhesive (280A). The seven-day tapes were prepared and counted by the methods previously described (Green et al., 2002). The data used correspond to the mean daily values expressed as the number of pollen grains per m^{-3} of air.

The meteorological data was obtained from the Australian Bureau of Meteorology, Queensland weather station at Brisbane airport, which is situated 10 kilometres north-east of the sampling site. The meteorological data included daily readings of maximum temperature, minimum temperature and total rainfall. Other readings including humidity, wind speed, wind direction, hours of sunshine were not available for the entire sampling period.

Data corresponding to five years (June 1994–May 1999) of pollen monitoring and meteorological records were documented graphically and evaluated statistically. To calculate the period of each individual ANAP season, the criteria developed by Nilsson and Persson (Nilsson and Persson, 1981) was used where the date when 5% of the total accumulated pollen count for that year corresponded to the beginning of the season and 95% of the total accumulated pollen count corresponded to the end of the season. Daily counts were classified into two categories (1–10, 11–100 grains m^{-3}), a standard which is used by the Palynological Laboratory, Swedish Museum of Natural History, Stockholm. Associations between mean daily ANAP counts and meteorological values were calculated using Spearman's (non-parametric) correlational analysis for the entire study period and by season. Statistical linear regressions relating meteorologic parameters to different aspects of ANAP aerobiology (start, length and intensity of the pollen seasons) were performed using Stata (Stata, 1999) to obtain an initial predictive model for forecasting the start and intensity of different ANAP seasons.

3. Results

3.1. Australian native arboreal pollen counts

ANAP is present in the atmosphere of Brisbane throughout the entire year with peak average daily counts occurring in the months May–November (Table 1, Figure 2) and total yearly counts of 851–1596 grains m^{-3} (Green et al., 2002; Table 2). Highest frequencies of Cupressaceae and Myrtaceae pollen occur during spring (August–November), whereas Casuarinaceae pollen frequencies peak during the winter months, May–September. The duration of the pollen season for each family varied from 65–171 days for Cupressaceae, 263–342 days for Casuarinaceae and 202–301 days for Myrtaceae with a mean seasonal duration of 98, 320 and 258 days for Cupressaceae, Casuarinaceae and Myrtaceae, respectively. Start dates of the ANAP seasons over the five-year period occurred during the interval; 8th–16th June for Casuarinaceae, 2nd–27th July for Myrtaceae and 8th–19th August for Cupressaceae (Table 1). End dates of ANAP seasons were variable ranging between October–January for Cupressaceae, March–May for Casuarinaceae and December–May for Myrtaceae. Daily ANAP counts were within the range of 11–100 grains m^{-3} on 100 days and between 1–10 grains m^{-3} on 1011 days during the five-year sampling period (Table 1).

3.2. Meteorology and Australian native arboreal pollen counts

Peak pollen emissions of ANAP in Brisbane coincided with average monthly maximum temperatures of 23–27 °C, minimum temperatures of 14–17 °C and rainfall less than 6 mm recorded for the region (Figures 2 and 3). The five-year ANAP counts plotted against maximum temperature, minimum temperature

Table 1. Seasonal distribution of Cupressaceae, Casuarinaceae and Myrtaceae pollen in Brisbane, June to May, 1994–1999. The seasonal dates where the accumulated sum since the beginning of the year reaches 5% and 95%, the peak spore count (grains m^{-3}) and date, seasonal duration and daily count categories are shown in each case

Pollen taxa	1994–1995	1995–1996	1996–1997	1997–1998	1998–1999
<i>Cupressaceae</i>					
5%	8/8	19/8	17/8	11/8	9/8
Peak Day	5/9	7/11	2/9	4/9	14/9
Peak Count	18.1	63.6	61.6	38.1	93.6
95%	25/1	26/11	20/10	30/10	20/10
Season Duration	171	100	65	81	73
Days count 11–100	5	16	19	9	20
Days count 1–10	90	39	51	64	63
<i>Casuarinaceae</i>					
5%	16/6	17/6	12/6	16/6	8/6
Peak Day	9/9	13/8	24/8	24/6	16/8
Peak Count	37.9	19.9	31.2	40.4	50
95%	5/3	19/5	19/5	20/5	21/4
Season Duration	263	338	342	339	318
Days count 11–100	3	4	3	4	14
Days count 1–10	101	60	79	98	72
<i>Myrtaceae</i>					
5%	2/7	9/7	19/6	27/7	8/6
Peak Day	24/10	5/11	11/10	23/9	9/8
Peak Count	12.1	4.1	18.8	7.7	7.7
95%	19/3	16/4	19/2	23/5	26/12
Season Duration	261	283	246	301	202
Days count 11–100	2	0	1	0	0
Days count 1–10	136	31	48	25	54

and rainfall illustrates fluctuations from one year to the next (Figure 3). Individually, pollen seasons of the Cupressaceae were comparatively homogeneous in duration, timing and intensity, whereas the pollen seasons of Casuarinaceae and Myrtaceae were heterogeneous with respect to daily pollen loads and seasonal duration. Inter-seasonal variations in the amount of airborne Casuarinaceae and Myrtaceae pollen may be attributed to daily rainfall, different species releasing their pollen at varying intervals, long distance pollen transport, post seasonal pollen refloating or entomophylly.

Non-parametric correlation analysis indicated strong negative associations ($p < 0.0001$) between daily Cupressaceae and Casuarinaceae seasonal counts and maximum and minimum temperature (Table 3) for each of the sampling years. Insignificant correlations between daily seasonal Myrta-

ceae counts and maximum and minimum temperature were evident, particularly for the periods 1994–1995, 1995–1996 and 1997–1998. Associations with rainfall were significant ($p < 0.05$) and negatively correlated with seasonal Cupressaceae and Casuarinaceae pollen counts. However, significant correlations for daily pollen loads of Myrtaceae were not as consistent, particularly during the period 1995–1998 (Table 3). Significant negative associations ($p < 0.0001$) were evident between maximum temperature, minimum temperature, rainfall and total ANAP counts for the entire sampling period 1994–1999 (Table 2).

The intensity of the Cupressaceae pollen season plotted against average pre-seasonal precipitation (Figure 4) showed a trend towards lower seasonal pollen loads (< 500 grains m^{-3}) when average pre-seasonal precipitation was less than 1 mm, and higher seasonal pollen loads (> 500 grains m^{-3})

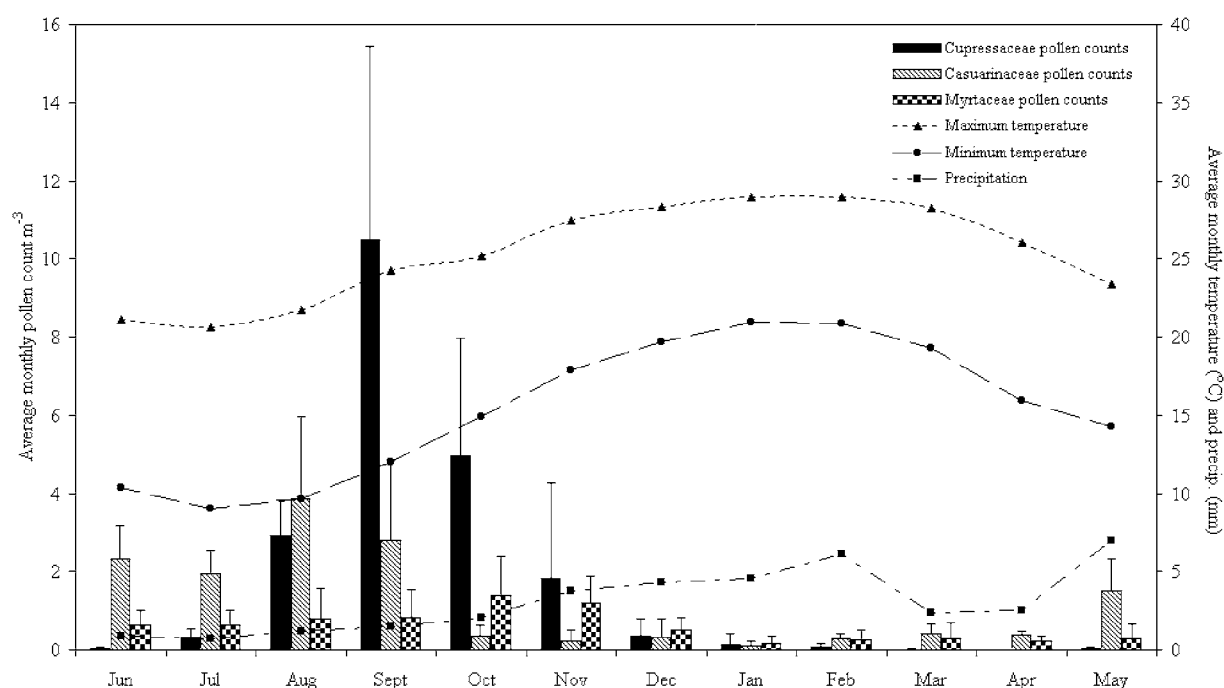


Figure 2. Average monthly Cupressaceae, Casuarinaceae and Myrtaceae pollen counts ($\pm$ SD) based on five sampling years (June 1994–May 1999) of daily pollen counts (grains m^{-3}) plotted with maximum temperature $^{\circ}\text{C}$, minimum temperature $^{\circ}\text{C}$ and rainfall (mm).

Table 2. Annual totals (grains m^{-3}) of daily pollen concentrations for the families Cupressaceae, Casuarinaceae and Myrtaceae and average total pollen counts for the period 1994–1999

Pollen taxa	Total annual count					Average 1994–1999
	1994–1995	1995–1996	1996–1997	1997–1998	1998–1999	
Cupressaceae	398	462	698	430	850	567
Casuarinaceae	475	265	344	445	582	422
Myrtaceae	419	124	200	125	164	206
Total	1292	851	1242	1000	1596	1196

when average pre-seasonal precipitation was greater than 1 mm. Statistical analysis of this relationship (Figure 4) supported that it was significant ($r^2 = 0.76$, $p = 0.05$). Moreover, r^2 values for maximum temperature ($r^2 = 0.47$) and minimum temperature ($r^2 = 0.65$) were obtained, but these were determined not to be statistically significant ($p > 0.1$).

A relationship was also obtained between the number of days to the start of the Myrtaceae season and the average maximum temperature from 1 June to the beginning of the season (Figure 5). Lower average pre-seasonal maximum temperatures (1994–1995, 1995–1996 and 1997–1998) coincided with later start dates for the Myrtaceae pollen season,

whereas earlier Myrtaceae seasons coincided with higher average pre-seasonal maximum temperatures (1996–1997 and 1998–1999); values were statistically significant ($r^2 = 0.78$, $p = 0.04$). Trends with minimum temperature ($r^2 = 0.44$) and rainfall ($r^2 = 0.02$) were not statistically significant ($p > 0.1$). Relationships between total seasonal Casuarinaceae/Myrtaceae frequencies and weather parameters prior to the season were also investigated and determined not to be statistically significant (data not shown).

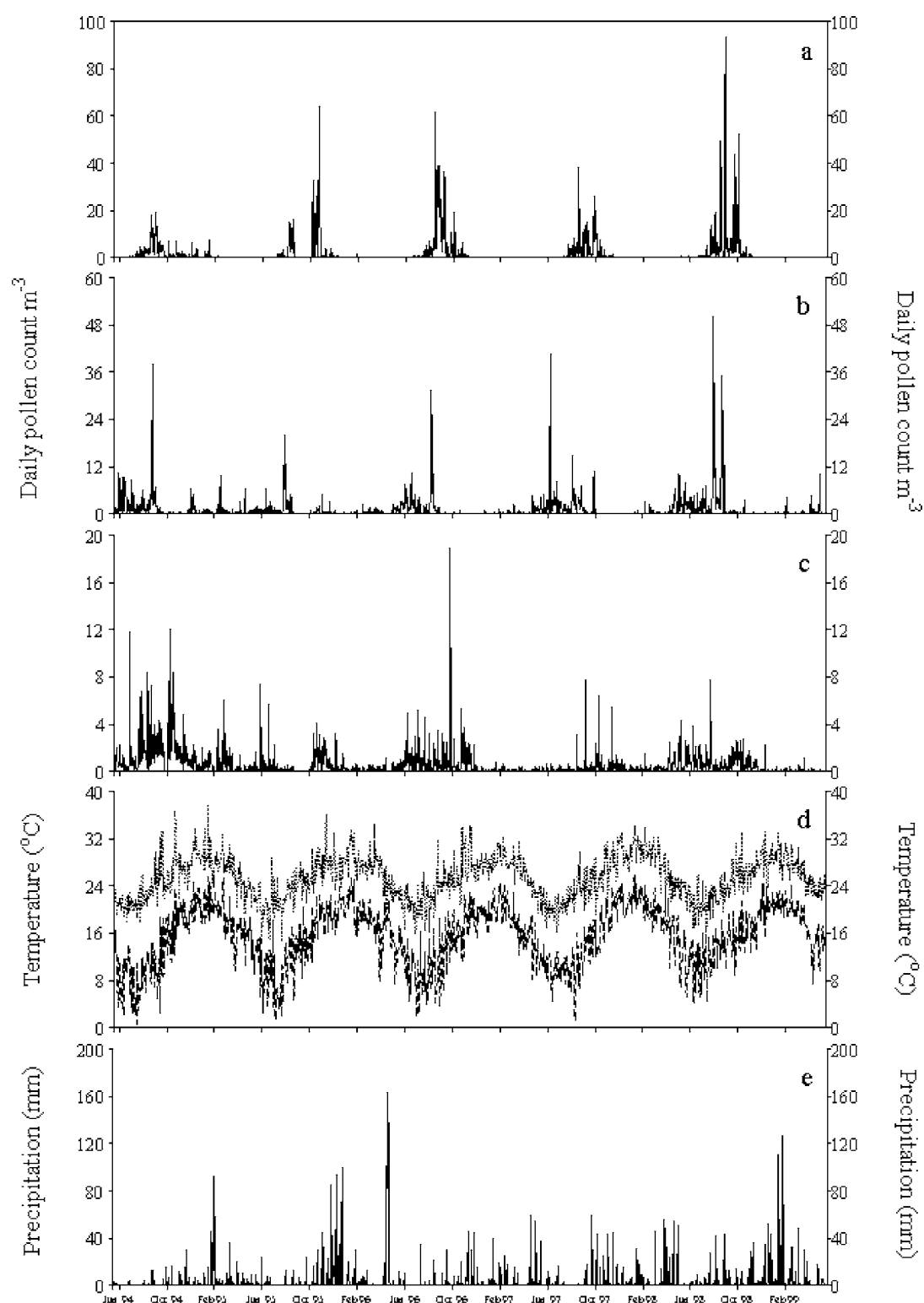


Figure 3. Seasonal distribution of (—) a. Cupressaceae, b. Casuarinaceae and c. Myrtaceae pollen in the atmosphere of Brisbane, Australia during five individual years plotted with daily (· · · · ·) d. maximum and (— — —) minimum temperature ($^{\circ}\text{C}$) and (—) e. total daily rainfall (mm).

Table 3. Spearman's correlation coefficients (r_s) between meteorological parameters and Cupressaceae, Casuarinaceae and Myrtaceae yearly ($n = 365$) and total pollen counts ($n = 1826$)

	r_s		
	$T_{\max}$	$T_{\min}$	Precip.
<i>Cupressaceae</i>			
1994–1995	0.1290*	–0.1409*	–0.1982*
1995–1996	–0.0154	–0.1418*	–0.0519
1996–1997	–0.2906**	–0.4791**	–0.1375*
1997–1998	–0.2465**	–0.4037**	–0.1761*
1998–1999	–0.4288**	–0.5089**	–0.1932*
1994–1999	–0.1763**	–0.3363**	–0.1557**
<i>Casuarinaceae</i>			
1994–1995	–0.3291**	–0.4238**	–0.1535*
1995–1996	–0.3601**	–0.4845**	–0.1504*
1996–1997	–0.5734**	–0.5979**	–0.1943*
1997–1998	–0.5410**	–0.6575**	–0.2218**
1998–1999	–0.5724**	–0.6397**	–0.1814*
1994–1999	–0.4792**	–0.5586**	–0.1873**
<i>Myrtaceae</i>			
1994–1995	0.1036	–0.1602*	–0.1122*
1995–1996	0.1942*	0.0659	–0.0341
1996–1997	–0.2883**	–0.4331**	–0.0984
1997–1998	0.0954	–0.0144	–0.0557
1998–1999	–0.3208**	–0.4669**	–0.1822*
1994–1999	–0.0634**	–0.2144**	–0.1099**

Significant r_s values; * $p < 0.05$; ** $p < 0.001$.

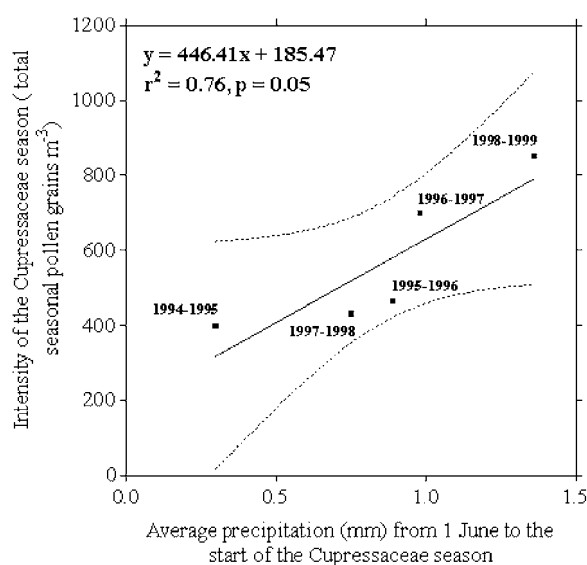


Figure 4. Intensity of the Cupressaceae season, established from the average pre-seasonal precipitation from 1 June against the total seasonal Cupressaceae pollen counts.

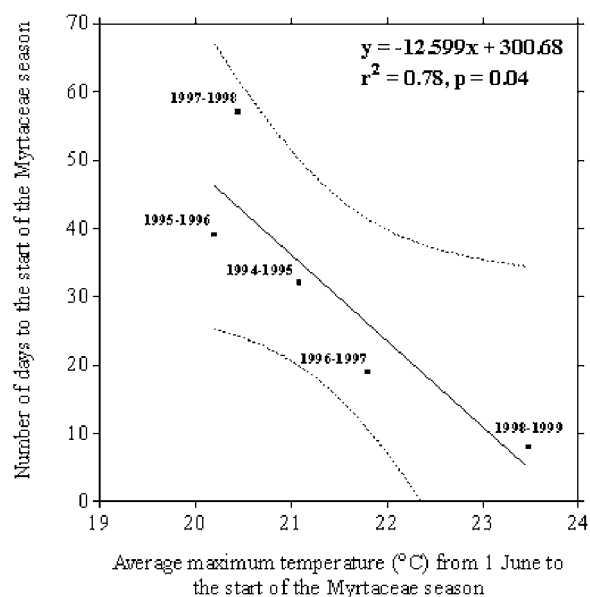


Figure 5. Start of the Myrtaceae season, established from the average pre-seasonal maximum temperature °C from 1 June against the number of days to the start of the Myrtaceae season.

4. Discussion

Pollen sourced from Australian native taxa belonging to the families Cupressaceae, Casuarinaceae and Myrtaceae are represented in the atmosphere of Brisbane throughout the entire year, contributing 18.4% to the annual pollen spectrum (Green et al., 2002). Maximum ANAP emissions are restricted to winter and spring, during the months May–November when rainfall and temperature values are at their lowest. ANAP seasons recorded in temperate regions of Australia are of comparable duration to those of Brisbane, but seasonal loads in temperate regions are significantly higher than in Brisbane. In Sydney, tree pollen comprised 65% of the total airborne count with Cupressaceae pollen the most frequent arboreal pollen type with highest concentrations (1842 grains m^{-3}) restricted to the months August and September (Bass and Morgan, 1997). Casuarinaceae and Myrtaceae pollen loads in Brisbane are similar in temporal distributions and total seasonal abundances (6.5%) to those recorded in Sydney, however daily peak loads are significantly lower in Brisbane. The Brisbane ANAP seasons are temporally comparable to those recorded in other Southern Hemisphere locations including Santiago, Chile (Rojas Villegas and Roure Nolla, 2001) and Johannesburg, South Africa (Cadman, 1990), by contrast to the Northern Hemisphere city

of Philadelphia, Pennsylvania where Cupressaceae pollen is only dispersed in the atmosphere for one month (Dvorin et al., 2001). Such differences in the duration and annual abundance of ANAP types can be attributed to localized variations in climatic factors as well as differences in taxa represented in the different regions.

Weather conditions, including maximum and minimum temperature were demonstrated in the statistical analyses to be significantly associated with the onset of the Myrtaceae pollen season and with daily Casuarinaceae and Myrtaceae pollen counts. Analogous associations with temperature variables and daily Casuarinaceae pollen counts have been established in Spain (Garcia et al., 1997). This study demonstrates for the first time statistically significant relationships linking pre-seasonal temperature to the onset of the Myrtaceae season in addition to associations between weather parameters and daily Myrtaceae concentrations. The variability between daily Myrtaceae pollen counts and weather parameters can be attributed to multi vector dispersal of its pollen, although the results demonstrate that Myrtaceae species depend on specific temperature regimes that lead to flower bud initiation and pollen anthesis. Previous studies have shown that cool temperatures, high amounts of rainfall and solar radiation prior to budding are all strong predictors for Myrtaceous flowering (Law et al., 2000; Moncur, 1992). However, difficulties differentiating between pollen of Myrtaceae taxa are well documented and should be taken into account when evaluating these results.

Precipitation, although not associated with daily Myrtaceae pollen counts, proved to be statistically associated with daily counts of Cupressaceae and Casuarinaceae pollen. Our findings also demonstrate a near-significant ($p = 0.05$) relationship between the average pre-seasonal precipitation and the annual Cupressaceae pollen loads for each season, with higher frequencies of seasonal pollen produced when average pre-seasonal precipitation was greater than 1 mm. Similar associations with precipitation have been identified for Cupressaceae in studies conducted in Denver, Colorado (Glassheim et al., 1995) and Spain (Garcia et al., 1997). Other meteorological parameters including temperature, humidity, and hours of sunshine are known to also influence daily and seasonal Cupressaceae pollen counts (Ariano et al., 1994; Galan et al., 1998; Kishikawa and Nagano, 1988; Yasuda et al., 1986).

Total yearly pollen counts and season durations can also be influenced by long distance pollen transport. It is possible that under the right atmospheric conditions, ANAP could be transported long distances from other sources. Long distance airborne pollen transport has been demonstrated for Cupressaceae in Tulsa, Oklahoma (Rogers and Levetin, 1998), Myrtaceae in Tasmania, Australia (Shimeld and Colhoun, 2001) and for Casuarinaceae in Macquarie Island, Australia (Salas, 1983). Cupressaceae pollen is often transported from central Texas to Oklahoma when the wind direction is favourable (Rogers and Levetin, 1998), while Myrtaceae and Casuarinaceae pollen is believed to have travelled from south-eastern Australia to the sub-antarctic, Macquarie Island during the Holocene period (Salas, 1983). These small, light pollen grains are capable of traveling thousands of kilometers from their point of origin, by entering the upper atmospheric jet streams, in which they are able to contribute to the aerobiological load of another geographical location.

Minimal amounts of daily ANAP were trapped on days when rainfall was recorded suggesting the removal or washing out of pollen from the atmosphere. Atmospheric pollen removal is well recognized (Hjelmroos, 1997; McDonald, 1962), however it is not singly associated with precipitation but to additional environmental parameters including wind speed and wind direction. Upon deposition, a pollen grain may be resuspended or refloat into the atmosphere following turbulent climatic events, including wind gusts. The refloating of pollen grains has been discussed previously for *Pinus* species (Green et al., 2003; Igarashi, 1987), however the extent to which refloating occurs for other pollen types is relatively unclear. In Sydney, Bass and Morgan (Bass and Morgan, 1997) concluded that the extended Casuarinaceae pollen seasons could be due to post-seasonal refloating of pollen. In this study refloating of ANAP grains occurred out of the season, particularly for Casuarinaceae pollen (data not shown) in very small concentrations and for short periods of time.

The seasonal incidence of allergy and respiratory disease caused by ANAP remains unclear and debatable in Australia. In spite of this, studies conducted in foreign regions where Australian native Casuarinaceae and Myrtaceae taxa have been introduced, demonstrate that the proportion of the population sensitised to ANAP is variable with prevalence rates ranging between 2.7–32.6% (Choonkook et al., 1998; Garcia et al., 1997; Mari et al., 1996; Wang and Chen, 1992). In Brisbane, personal exposure to ANAP con-

centrations high enough to elicit respiratory symptoms are restricted to a brief period of the Cupressaceae season each year during the months August–November. Moreover, low frequencies of Casuarinaceae and Myrtaceae pollen are distributed annually and this presents a constant allergen exposure risk, particularly as recent proteomic studies have shown that Australian native species share cross reactive allergens with the allergenically significant species *Cupress sempervirens* and *Paspalum notatum* (Hunter et al., 1996; Pham et al., 1994).

5. Conclusions

Results of the study demonstrate that ANAP is distributed throughout the entire year in relatively low concentrations, with maximum pollen emissions coinciding with the Cupressaceae season during the months, August–November. Daily concentrations of Cupressaceae and Casuarinaceae pollen were significantly associated with weather parameters. Associations between daily Myrtaceae pollen and weather parameters were heterogeneous and can be attributed to multiple dispersal vectors in which pollen of this family disseminates into the atmosphere. A relationship between the intensity of the Cupressaceae pollen season and pre-seasonal precipitation was established, with the seasons of greater intensity occurring when average pre-seasonal precipitation was greater than 1 mm. Additionally, the number of days to the start of the Myrtaceae season was found to be significantly related to the pre-seasonal average maximum temperature, with earlier seasons occurring when the average pre-seasonal temperature was greater than 21 °C. Personal exposure to elevated levels of ANAP occur over short intervals each year with a total of 100 days recorded over the five-year sampling period where pollen frequencies varied between 11–100 grains m⁻³. A larger data set combined with a parallel phenological study and accompanied by a comprehensive characterization of pollen belonging to Myrtaceae taxa may provide more insight into the effect of meteorological parameters on anthesis and the daily and seasonal pollen prevalence. To date, the incidence of allergy to ANAP throughout Australia remains unclear and requires further investigation.

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