

Fern spore and pollen airspora profile of Singapore

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Abstract Monitoring atmospheric fern spore and pollen loads in Singapore was initiated in June 1990. Aside from the more numerous fungal spores, fern spores and pollen grains made up 6.2–8.6% and 4.4–5.4% of the total airspora sampled, respectively. The most frequently encountered fern spores, in descending order, were those of *Nephrolepis auriculata*, *Dicranopteris linearis*, *Stenochlaena palustris*, *Asplenium nidus*, *Pteridium aquilinum*, and *Dicranopteris curranii*. For pollen grains, the most frequently encountered, in descending order, were *Elaeis guineensis*, *Casuarina equisetifolia*, *Acacia auriculiformis*, *Kyllingia polyphylla*, *Podocarpus*, and Poaceae pollen grains. Seasonal patterns for individual fern spore or pollen types were discernible despite the relatively uniform tropical climate in Singapore. The fern spore and pollen calendar for the period 1991–1995 was compared to that of 2005–2006 as a follow-up study to keep abreast with the rapidly changing landscape of Singapore. Diurnal patterns showing a late morning to afternoon peak

period were seen in fern spores, while the peak period was in the morning for pollen types studied with the exception to oil palm pollen. Additionally, association between fern spore and pollen counts and local meteorological conditions were also analyzed and found to be highly correlated. This study has thus identified the fern spores and pollen airspora components, and determined the calendars, as well as diurnal profiles of the Singapore airspora and provides invaluable information for allergy studies by highlighting the trigger sources present in the environment.

Keywords Fern spores and pollen · Calendar · Diurnal pattern · Tropical country · Meteorological factors · Singapore

1 Introduction

The term “airspora” was first used by Gregory and Hirst (1957) to describe the population of airborne particles of biological origin. The meaning of the term has evolved and is now commonly being used to describe airborne pollen and fungal spores (Mandrioli et al. 1998; Burge 1986). The concentrations of airborne pollen or spores and durations of exposure to these allergens have been found to be important factors influencing the exacerbation of allergic

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diseases. The constituents of airspora, such as fungal spores and pollen, have long been associated with increase of symptoms of allergic disease and asthma (Rossi et al. 1993; Zwick et al. 1991; Salvaggio et al. 1971). A Singapore study has also demonstrated positive skin prick reactions to local fern spore and pollen in allergic individuals (Chew et al. 2000). Studies in neighboring Malaysia and Indonesia also found sensitization to grass and tree pollen, and fern spores that make up the local airspora (Baratawidjaja et al. 1999; Sam et al. 1998).

The airspora in Southeast Asia, a tropical region, has not been well reported. Trees and shrubs flourish year-round with an abundance of ferns (Turner et al. 1997; Appanah 1993; Sim et al. 1992; Corlett 1990). There is no distinct definable major flowering season for plants (Brearley et al. 2007; Appanah 1985) as compared to temperate countries, resulting in unique airspora profiles that mirror the local vegetation. Airspora studies have been restricted mainly to the mid- to late-1990s which reported the predominance of fungal spores year-round and which constitute more than 83% of total airspora while the rest consists of fern spores and pollen (Lim et al. 1998; Sam et al. 1998; Ho et al. 1995).

This paper aims to describe the fern spore and pollen profiles in Singapore based on a survey from 1991 to 1995. The country has had more development through the years resulting in an ever-changing landscape, so a follow-up study was initiated in 2005–2006. A comparison of airspora profiles from 1991 to 1995 (60 months) and 2005–2006 (24 months) was made to determine the influence of the changing landscape on the airspora profiles.

2 Methods

2.1 Study area

Singapore is a city-state located at the southern tip of the Malay Peninsula at 1° 19' North and 103° 31' East between Malaysia and Indonesia. Its total land area as at 2010 was 712.4 km², with the highest point being Bukit Timah at 166 m above sea level (<http://www.singstat.gov.sg/stats/keyind.html>). About 81.6% of its 4.83 million population lives in high-rise buildings (Housing Development Board 2009). The climate is characterized by a generally uniform pressure,

temperature, high humidity, and abundance of rain-fall throughout the year (National Environment Agency Singapore 2007). The climate is marked by two distinct monsoon seasons, December to March (Northeast monsoon) and June to September (Southwest monsoon). The driest period from May to July while the wettest period is from November to January. Humidity often exceeds 90% at night till dawn, falling to 60% around midday with average daily humidity at 84.2%.

2.2 Sampling details

Air sampling was conducted using the Hirst-type volumetric spore trap (Hirst 1952). The traps were set up on the rooftops in three locations at Clementi (1° 18' 55.8'' N, 103° 46' 5.9'' E), Hougang (1° 21' 28.4'' N, 103° 53' 18.1'' E), and Kent Ridge (1° 17' 44.8'' N, 103° 46' 44.2'' E) (Fig. 1). Sampling was conducted in Kent Ridge and Clementi from 1995 while that for Hougang was only initiated in 1993. During 2005–2006, only the trap at the Kent Ridge campus site was maintained. For this reason, it has been possible to compare 1991–1995 and 2005–2006 data only in this sampling point. The traps were located 61, 28, and 45 m above sea level or 57, 27, and 44 m above ground, respectively. The Kent Ridge sampling site is at the fringe of a vegetation patch dominated by an anthropogenic secondary heath forest that grows on degraded soil known locally as *adinandra* belukar (Sim et al. 1992) located on a ridge, while the



Fig. 1 Location of sampling sites

Clementi and Hougang sampling sites are urban townships dominated by managed vegetation consisting primarily of roadside trees, turfed areas, and landscaped gardens (Fig. 1).

The trap consists of a tape wound around a drum coated with silicon vacuum grease (Beckman Instruments Inc., USA, catalogue no. 335149). Rotating at 2 mm per hour, the drums were changed weekly. The tapes were then cut at the 12 am line, corresponding to the 7 days of the week, and laid onto glass slides. Meteorological data for Kent Ridge and Clementi were obtained from the on-site meteorological stations, while Hougang's were interpolated from Seletar—the nearest sited Meteorological Services Division, National Environmental Agency (NEA) meteorological station. Subsequently, meteorological data for 2005–2006 were obtained from the weather station of the Department of Geography, National University of Singapore at Kent Ridge Campus on top of Block E2, Engineering Drive 1 (1° 18' N, 103° 6' E), owing to the close proximity of the station to the sampling site (940 m apart). The slides were screened using continuous sweeps across three horizontal traverses (total area 64.8 mm²) at 400× magnification (field area diameter 0.45 mm) (Tan et al. 1992; Lim et al. 1998). The airspora load was reflected as the number of spores or pollen grains m⁻³ month⁻¹. Airspora were identified based on reference slides of the local airspora using a comparison microscope.

For the diurnal studies, tapes collected from the Kent Ridge station during the months of March, April, and June from year 1995, 1996, and 1997 were examined. These months were analyzed owing to overlapping seasonal periods for all airspora types. The 12 vertical traverses screening method was adopted for the diurnal pattern study. The magnification of 400× was also used in this part of the study. Diurnal patterns were obtained by using the average from hourly counts. Days where no major fern spores and pollen of interest were counted were not used. The airspora load for diurnal studies was reflected as the percentage of spores or pollen grains per day.

2.3 Analysis

Comparisons were made between the fern spores and pollen captured at the three sampling stations. Percentages were used to reflect the seasonality profiles of the fern spores and pollen owing to the variability in

airspora densities between years. Effects of meteorological variables on daily and hourly airspora load were also studied. Spearman's rank correlation test was used for comparison between sampling stations and the yearly seasonal patterns. Effects of meteorological variables on fern spores and pollen were also analyzed by Spearman's correlation. All statistical analyses were performed using the PASW statistical package for Microsoft Windows (PASW version 18, USA).

3 Results

3.1 Airspora components

Apart from fungal spores (Lim et al. 1998), the outdoor airspora of Singapore consists of between 6.2 and 8.6% fern spores, and 4.4–5.4% pollen grains. In total, 34 fern spore types and 51 pollen types were identified through the years of sampling (Table 1). However, fern spores and pollen grains that make up less than 5% of the total fern spores or total pollen were categorized as “others.”

Spores of *Nephrolepis auriculata* were the most abundant fern spores in the airspora (Table 2) followed by *Dicranopteris linearis*, *Stenochlaena palustris*, *Asplenium nidus*, *Pteridium aquilinum*, and *Dicranopteris curranii*. The ranking of spore types (by spore density) was similar at all three sites with minor exceptions. *Pteridium aquilinum* and *Dicranopteris curranii* were ranked higher than *Asplenium nidus* in Clementi and Hougang compared to Kent Ridge. Additionally, only 0.3% of the fern spores trapped in this study were unidentifiable.

Pollen from casuarina (*Casuarina equisetifolia*) was the most abundant pollen type in the atmosphere at Kent Ridge followed by the oil palm (*Elaeis guineensis*) pollen and *Acacia auriculiformis* (Table 2). However, at Clementi and Hougang, oil palm pollen was the most abundant, followed by pollen of *Kyllingia polyphylla*. However, *Podocarpus* pollen was found at a higher density than *Acacia auriculiformis* at Clementi. In general, oil palm and *Acacia auriculiformis* pollen grains were trapped on more days in Kent Ridge and Clementi, which are located to the south of the island, while oil palm and *Kyllingia polyphylla* pollen were found in the trap on more days at Hougang, which is located northeast of Singapore. Poaceae and Palmae pollen, which occur

Table 1 Fern spores and pollen in the Singapore airspora between 1991–1995 at three sampling sites (Kent Ridge, Clementi, and Hougang), and 2005–2006 at Kent Ridge

Spores
Common spores (trapped > 5% of total sampling days surveyed)
<i>Asplenium nidus</i> L. (Aspleniaceae)
<i>Dicranopteris curranii</i> Copel. (Gleicheniaceae)
<i>Dicranopteris linearis</i> (Burm.f.) Underw. (Gleicheniaceae)
<i>Nephrolepis auriculata</i> (L.) Trimen/N. <i>falcata</i> (Cav.) C.Chr./N. <i>radicans</i> (Burm.f.) Kuhn (Oleandraceae)
<i>Ophioglossum reticulatum</i> L. (Ophioglossaceae)
<i>Pteridium aquilinum</i> (L.) Kuhn (Dennstaedtiaceae)
<i>Pyrrosia piloselloides</i> (L.) M.G. Price (Polypodiaceae)
<i>Stenochlaena palustris</i> (Burm.f.) Bedd. (Blechnaceae)
Other spores (trapped on less than 5% of total sampling days surveyed)
<i>Adiantum latifolium</i> Lam. (Adiantaceae)
<i>Adiantum malesianum</i> Ghatak (Adiantaceae)
<i>Adiantum trapeziforme</i> L. (Adiantaceae)
<i>Blechnum orientale</i> L. (Blechnaceae)
<i>Ceratopteris thalictroides</i> (L.) Brongn. (Pakeriaceae)
<i>Cyathea latebrosa</i> (Wall. ex Hook.) Copel. (Cyatheaceae)
<i>Cyclosorus sumatranus</i> (Alderw.) Ching (Thelypteridaceae)
<i>Davallia denticulata</i> (Burm.f.) Mett. ex Kuhn (Davalliaceae)
<i>Drynaria quercifolia</i> (L.) J.Sm./D. <i>sparsisora</i> (Desv.) T.Moore (Polypodiaceae)
<i>Goniophlebium percutum</i> (Cav.) W.H. Wagner & Grether (Polypodiaceae)
<i>Lindsaea</i> spp. (Dennstaedtiaceae)
<i>Lygodium flexuosum</i> (L.) Sw. (Schizaeaceae)
<i>Lygodium microphyllum</i> (Cav.) R.Br. (Schizaeaceae)
<i>Nephrolepis multiflora</i> (Roxb.) Jarrett (Oleandraceae)
<i>Phyllitis scolopendrium</i> (L.) Newman (Aspleniaceae)
<i>Phymatosorus scolopendria</i> (Burm.f.) Pic.Serm. (Polypodiaceae)
<i>Pityrogramma calomelanos</i> (L.) Link (Adiantaceae)
<i>Platycerium coronarium</i> (D. Koenig ex O.F. Müll.) (Polypodiaceae)
<i>Pteris ensiformis</i> Burm.f. (Pteridaceae)
<i>Pteris vittata</i> L. (Pteridaceae)
<i>Pyrrosia lanceolata</i> (L.) Farwell (Polypodiaceae)
<i>Pyrrosia longifolia</i> (Burm.) C.V. Morton (Polypodiaceae)
<i>Selaginella</i> species (Selaginellaceae)
<i>Taenitis blechnoides</i> (Willd.) Sw. (Adiantaceae)
<i>Vittaria elongata</i> Sw./V. <i>ensiformis</i> Sw. (Vittariaceae)

Table 1 continued

Pollen
Common pollen (trapped > 5% of total sampling days surveyed)
<i>Acacia auriculiformis</i> A.Cunn. ex Benth. (Mimosaceae)
<i>Casuarina equisetifolia</i> J.R. & G. Forst. (Casuarinaceae)
<i>Elaeis guineensis</i> Jacq. (Palmae)
<i>Kyllingia polyphylla</i> Willd. ex Kunth (Cyperaceae)
<i>Pinus</i> spp./ <i>Podocarpus</i> spp. (Podocarpaceae)
Poaceae
Palmae (excluding <i>Elaeis guineensis</i>)
Other pollen (trapped on less than 5% of total sampling days surveyed)
<i>Acacia mangium</i> Willd. (Mimosaceae)
<i>Adinandra dumosa</i> Jack (Theaceae)
<i>Amaranthus</i> spp. (Amaranthaceae)
<i>Archidendron clyperia</i> (Jack) I.C. Nielsen/ <i>Paraserianthes falcata</i> (L.) I.C. Nielsen (Leguminosae)
<i>Archidendron jiringa</i> (Jack) I.C. Nielsen (Leguminosae)
<i>Archontophoenix alexandrae</i> (F.J. Muell.) H.A. Wendl. & Drude. (Palmae)
<i>Caesalpinia pulcherrima</i> (L.) Sw. (Caesalpinaceae)
<i>Calliandra emarginata</i> (Humb. & Willd.) Benth. (Mimosaceae)
<i>Callistemon citrinus</i> (Curtis) Skeels. (Myrtaceae)
<i>Cassia fistula</i> L. (Caesalpinaceae)
<i>Coccoloba uvifera</i> (L.) L. (Polygonaceae)
<i>Complaya trilobata</i> (L.) Strother (Compositae)
<i>Cordia cylindrostachya</i> (Ruiz & Pav.) Roem. & Schult. (Boraginaceae)
<i>Cyperus rotundus</i> L. (Cyperaceae)
<i>Dillenia suffruticosa</i> (Griff.) Martelli (Dilleniaceae)
<i>Eclipta prostrata</i> (L.) L. (Compositae)
<i>Emilia sonchifolia</i> (L.) DC. (Compositae)
<i>Eugenia grandis</i> Wight/ <i>E. longiflora</i> (Presl) F.-Vill./ <i>E. spicata</i> Lamk. (Myrtaceae)
<i>Eugenia jambos</i> L. (Myrtaceae)
<i>Eugenia polyantha</i> Wight (Myrtaceae)
<i>Glochidion hypoleucum</i> (Miq.) Boerl. (Euphorbiaceae)
<i>Hevea brasiliensis</i> (Willd. ex A. Juss.) Müll. Arg. (Euphorbiaceae)
<i>Lagerstromia indica</i> L. (Lythraceae)
<i>Lawsonia inermis</i> L. (Lythraceae)
<i>Macaranga heynei</i> I.M. Johnst. (Euphorbiaceae)
<i>Mallotus paniculatus</i> (Lam.) Müll. Arg. (Euphorbiaceae)
<i>Melaleuca cajuputi</i> Powell/ <i>M. leucandendra</i> (L.) L./ <i>M. viridiflora</i> Sol. ex Gaertn. (Myrtaceae)

Table 1 continued

Pollen
<i>Melastoma malabathricum</i> L. (Melastomataceae)
<i>Mimosa pigra</i> L. (Mimosaceae)
<i>Mimosa pudica</i> L. (Mimosaceae)
<i>Muntingia calabura</i> L. (Muntingiaceae)
* <i>Morella esculenta</i> (Buch.-Ham. ex D.Don) I.M. Turner (Myricaceae)
<i>Peltophorum pterocarpum</i> (DC.) K. Heyne (Caesalpiniaceae)
<i>Pilea microphylla</i> (L.) Liebm. (Urticaceae)
* <i>Plantago major</i> L. (Plantaginaceae)
<i>Rhodamnia cinerea</i> Jack (Myrtaceae)
<i>Ricinus communis</i> L. (Euphorbiaceae)
<i>Samanea saman</i> (Jacq.) Merrill (Mimosaceae)
<i>Sterculia monosperma</i> Vent. (Sterculiaceae)
<i>Theobroma cacao</i> L. (Sterculiaceae)
<i>Tridax procumbens</i> L. (Compositae)
<i>Typha angustifolia</i> L. (Typhaceae)
<i>Vernonia cinerea</i> (L.) Less. (Compositae)
New pollen types that were found in 2005–2006 are indicated with an asterisk

at more than 5% of the sampling days, were trapped at all three stations but at much lower densities. Other pollen grains consisting of 45 other pollen types were trapped with similar densities at all the sampling sites. Higher numbers of unidentified pollen types were trapped in this study.

3.2 Correlation between spore counts of different sites

Fern spore and pollen counts of all three surveyed sites were significantly correlated (Table 3). Stronger correlations were observed between Kent Ridge and Clementi as well as Kent Ridge and Hougang. Exceptions were seen in *Asplenium nidus* and *Dicranopteris curranii* between Clementi and Hougang. Pollen from casuarina, palm, and *Podocarpus* was not correlated between Clementi and Hougang, while no correlation was observed for Poaceae pollen between all sampling sites.

3.3 Composition of fern and pollen in airspora from the pilot and follow-up studies

Although airspora sampling was conducted from 1991 and is still ongoing at the Kent Ridge sampling

site, analysis of the samples could not be maintained because of manpower shortage. With rapid development experienced by Singapore and its ever-changing landscape, a follow-up study was carried out by analyzing samples collected from 2005 to 2006 to detect any changes in airspora makeup and trends after 10 years. Airspora composition from 1991 to 1995 and 2005–2006 is shown in Table 4. Fern spore types and percentages of contribution remained essentially similar. However, changes were observed in the pollen components in 2005–2006 as compared to 1991–1995. Contributions of casuarina, oil palm, and *Acacia auriculiformis* pollen grains were lower in 2005–2006. Furthermore, Poaceae pollen now ranked in the top three major contributors to the pollen component. A higher percentage of unidentified pollen types was also observed in 2005–2006. *Morella esculenta* and *Plantago major* pollen were new pollen types captured in the trap during 2005–2006 sampling period.

3.4 Seasonal variation

The seasonal variation for fern spores is illustrated in Fig. 2. Only the six major spore types that were frequently trapped are presented. Fern spores were found in the atmosphere perennially. However, the periods of high fern spore counts tended to stretch over 2–6 months. Nevertheless, the seasonal profiles of the individual spore types were observed to be relatively similar at all three sampling sites as seen by the highly correlated airspora counts (Table 3).

May–October is the period of high fern spore counts because of the overlapping seasons of the different fern types (Fig. 2). Spores of *Nephrolepis auriculata*, *Dicranopteris linearis*, and *Dicranopteris curranii* were elevated from May–October with highest densities observed in June–July. *Pteridium aquilinum* spores increase sharply in June, tapers slightly downwards before peaking again in September and tapering down as the year progresses. *Stenochlaena palustris* also showed a double-peak pattern of almost similar intensities in May and October–November. *Asplenium nidus* spores increase less sharply with highest counts usually in October–November. Seasonality profiles for *Nephrolepis auriculata*, *Dicranopteris linearis*, and *Stenochlaena palustris* in 1991–1995 and 2005–2006 were similar with the high correlation between yearly counts in

Table 2 Percentage contribution of individual fern spore and pollen types to the total counts at the three sampling stations from 1993 to 1995, respectively, and percentage number of days individual spore or pollen types occurred yearly, respectively

Spore or pollen types	Kent Ridge		Clementi		Hougang	
	% total spores or pollen	% days occurred	% total spores or pollen	% days occurred	% total spores or pollen	% days occurred
Fern						
<i>Nephrolepis auriculata</i>	50.9	80.4	54.9	85.3	55.8	90.6
<i>Dicranopteris linearis</i>	27.1	67.3	24.4	68.1	24.8	73.2
<i>Stenochlaena palustris</i>	6.2	35.0	5.2	37.5	5.2	42.4
<i>Asplenium nidus</i>	3.8	33.7	2.5	25.7	2.0	27.3
<i>Pteridium aquilinum</i>	3.5	22.2	3.6	24.1	2.8	26.1
<i>Dicranopteris curranii</i>	3.4	18.0	4.4	21.8	3.8	17.0
Other fern	4.8	45.5	5.2	45.3	5.3	52.1
Unknown fern	0.3	1.3	0.3	1.4	0.3	2.7
Gymnosperm/angiosperm						
<i>Casuarina equisetifolia</i> (casuarina)	28.0	24.4	14.7	17.4	7.2	14.5
<i>Elaeis guineensis</i> (oil palm)	25.1	35.5	23.6	39.6	45.3	46.6
<i>Acacia auriculiformis</i>	18.2	45.3	9.7	35.1	4.2	26.6
<i>Kyllingia polyphylla</i>	5.3	11.1	23.1	26.9	20.8	30.6
Palmae (excludes oil palm)	2.3	13.7	1.3	7.9	1.1	6.7
<i>Podocarpus</i>	2.3	13.5	15.5	25.9	1.6	8.3
Poaceae	2.2	6.7	3.4	9.0	3.3	8.0
Other pollen	14.3	34.9	6.7	19.4	7.3	19
Unknown pollen	2.4	6.7	2.0	6.3	9.2	14.4

Table 5. Fern spore types that occur at lower densities showed no correlation between their yearly correlation from 1991 to 1995 as well as with those of 2005–2006.

Generally, seasonal patterns of the pollen are marked with a sharper increase in airborne pollen grains in the air (Fig. 3). Seasonality also varies more frequently between the sampling years for pollen. Casuarina repeatedly demonstrated a double-peak seasonality (March–April and September). Oil palm was the only pollen type whose pollen grains were not captured in the trap from June–August, but later, the number of airborne pollen increases steadily to reach high counts in January–March. *Acacia auriculiformis* highest counts were recorded yearly in July. The following three pollen types were trapped at much lower frequencies. *Kyllingia polyphylla* pollen peak in April while *Podocarpus* pollen in November and February. Poaceae seasonality was varied with its highest month in March (1993 and 1994), June (1991), September (1995), and December (1991).

Oil palm and *Acacia auriculiformis* pollen counts were significantly correlated throughout all the studied years, indicating similar yearly seasonality patterns. Casuarina, *Kyllingia polyphylla*, *Podocarpus*, and Poaceae pollen counts were mostly not correlated.

3.5 Diurnal variations

The diurnal pattern for fern spores is shown in Fig. 4a. More variability in peak hours was seen in fern spores compared to any pollen type. The fern spores' peak hour occurs in the late morning and early afternoon with the exception of *Asplenium nidus* spore counts that peak earlier at around 9 am. A variable diurnal pattern was observed for *Stenochlaena palustris*. Double peaks were observed at 11 am and 5 pm in 1995 followed by continuously high counts from 7 am to 7 pm in 1996 and a single peak at 2 pm in 1997.

A more specific and distinct diurnal pattern was seen in the pollen counts (Fig. 4b). Generally,

Table 3 Correlation between fern spore and pollen counts at Kent Ridge, Clementi, and Hougang

Comparative sites: spore/ pollen type†	Kent Ridge versus Clementi	Clementi versus Hougang	Hougang versus Kent Ridge
Spearman's correlation coefficients (<i>p</i> -value)			
Fern			
<i>Nephrolepis auriculata</i>	0.29***	0.08*	0.19***
<i>Dicranopteris linearis</i>	0.30***	0.17***	0.24***
<i>Stenochlaena palustris</i>	0.25***	0.18***	0.23***
<i>Asplenium nidus</i>	0.12***	ns	0.08**
<i>Pteridium aquilinum</i>	0.38***	0.35***	0.35***
<i>Dicranopteris curranii</i>	0.17***	ns	0.13**
Gymnosperm/angiosperm			
<i>Casuarina equisetifolia</i> (casuarina)	0.32***	ns	0.21***
<i>Elaeis guineensis</i> (oil palm)	0.52***	0.59***	0.61***
<i>Acacia auriculiformis</i>	0.27***	0.22***	0.14***
<i>Kyllingia polyphylla</i>	0.07*	0.09**	0.14***
Palmae (excludes oil palm)	0.15***	ns	ns
<i>Podocarpus</i>	0.10***	ns	0.08*
Poaceae	ns	ns	ns

Spore/pollen types are listed in descending order based on the percentage contribution to the total counts at the Kent Ridge station ($n = 729$).

Correlation *p*-values:

* $p < 0.05$; ** $p < 0.01$;

*** $p < 0.001$

ns not significant

maximum pollen densities were observed in the mid-mornings to early afternoon. A broad peak was observed for casuarina pollen grain counts from 8 am to 2 pm. The pollen type with the latest peak hour was oil palm at 2 pm. Variable peak hours were observed for *Acacia auriculiformis* pollen grain counts falling between 6 am and 4 pm showing two peaks with the first higher than the second. A distinct diurnal pattern was observed only in 1995 (6 am) and 1996 (10 am) for the Poaceae.

3.6 Meteorological variables and airspora

Spearman's correlation was used to determine meteorological factors that are correlated with fern spore and pollen grain counts (Table 6). In general, fern spores were inversely correlated with rainfall and relative humidity with the exception of *Asplenium nidus*. Increased spore counts of *Nephrolepis auriculata*, *Dicranopteris linearis*, and *Dicranopteris curranii* were also seen with high temperature and total solar radiation coupled with low wind speed. Spores from *Stenochlaena palustris*, *Asplenium nidus*, and *Pteridium aquilinum* were also affected by high temperature and total solar radiation but not by wind speed.

All pollen counts were also inversely correlated with rainfall and relative humidity. Casuarina,

Kyllingia polyphylla, and grasses pollen counts also showed increases with high temperature, solar radiation, and wind speed. High wind speed, low temperature, and afternoon relative humidity also result in more oil palm pollen being trapped on the sites. *Acacia auriculiformis* densities were the only airspora type positively correlated with maximum temperature. *Podocarpus* pollen counts were also positively correlated with wind speed and negatively correlated with average and minimum daily temperature.

In diurnal counts, most of the fern spores and pollen grains were positively correlated with temperature and negatively with relative humidity (Table 7). Only *Nephrolepis auriculata* and oil palm showed weak but nevertheless negative correlation with rainfall. A strong correlation was also seen with hourly oil palm pollen counts and wind speed.

4 Discussion

Tropical climatic conditions make it favorable for plants to thrive throughout the year, resulting in a rich airspora comprising not only of pollen but fern spores, a result of the abundance of ferns in the tropics (Sim et al. 1992; Holtum 1968). In this study, fern spores were not only found to be present in the

Table 4 The annual contribution of individual fern spore or pollen types to the total fern spore or pollen counts, respectively, at the Kent Ridge station

Spore/pollen type	1991	1992	1993	1994	1995	2005	2006
Fern							
<i>Nephrolepis auriculata</i>	40.3	58	55.3	37	56.1	64.4	34.4
<i>Dicranopteris linearis</i>	35.7	23	21.3	32.9	26.4	13.6	21.2
<i>Stenochlaena palustris</i>	5.1	5.7	6.4	8.2	5.1	3.9	8.6
<i>Asplenium nidus</i>	3.2	3.5	4.5	4.7	5.2	1.5	10.1
<i>Pteridium aquilinum</i>	5.8	3	3.6	5.6	0.6	9.3	6.4
<i>Dicranopteris curranii</i>	6.2	2.6	2.1	3.8	3.3	5.0	10.8
Other fern	3.7	4.1	5.4	7.7	2.9	1.0	7.9
Unknown fern	<0.0	0.1	0.4	0.1	0.4	<0.0	<0.0
Gymnosperm/angiosperm							
<i>Casuarina equisetifolia</i>	41.7	35.4	27.2	18.5	22.1	16.3	10.3
<i>Elaeis guineensis</i>	15.8	28.5	24.9	20.9	44.8	18.4	12.0
<i>Acacia auriculiformis</i>	15.5	14	12.8	22.4	9.8	4.8	5.8
<i>Kyllingia polyphylla</i>	3.4	5.3	7	5.2	7.2	6.5	4.0
Palmae	0.8	0.4	2.4	6.2	3.4	1.8	3.5
<i>Podocarpus</i>	1.3	2.1	2.3	2.8	3	1.3	2.5
Poaceae	3.9	1.8	1.5	2.3	0.3	20.5	13.1
Other pollen	16.3	10.6	16.7	15.3	9	17.7	25.9
Unknown pollen	1.3	1.9	5.2	6.4	0.4	8.5	18.9

Only spore or pollen types that occurred more than 5% of the total number of days surveyed are listed. Spore or pollen types are listed in descending order based on the percentage contribution to the overall total counts at the station

environment year-round but also contributed a higher percentage to the total airspora component compared to pollen grains.

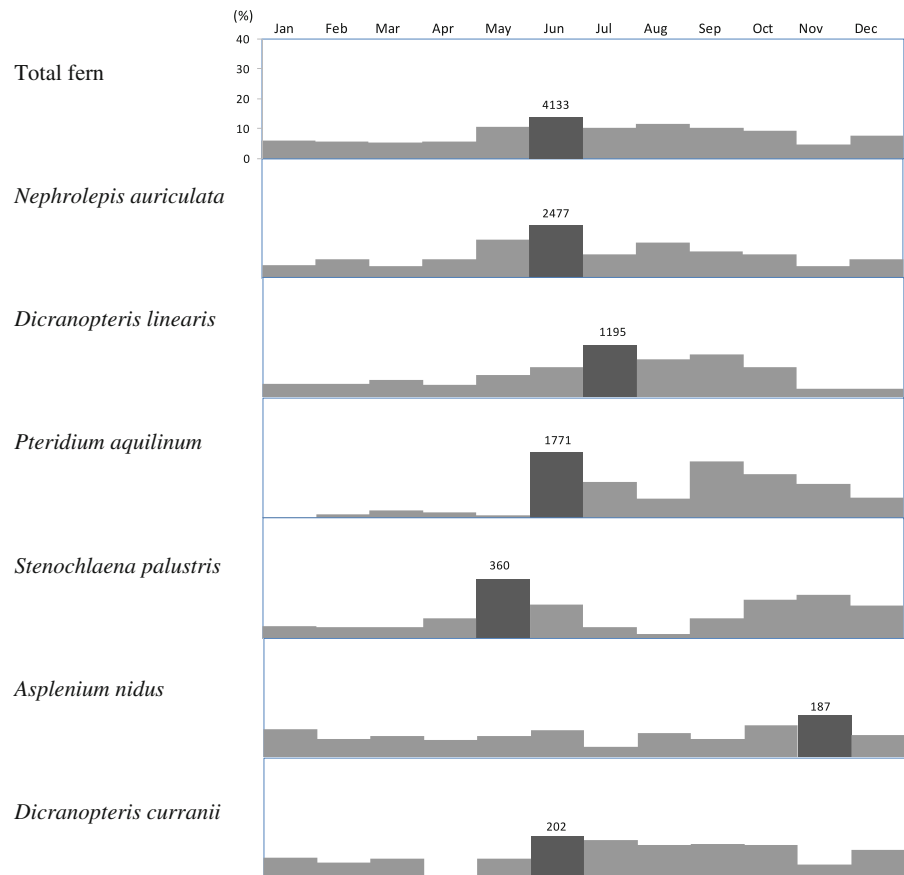
In Bangkok, Thailand, fern spores were found to be the third most common airborne airspora in the atmosphere after fungal spores and pollen (Bunnag et al. 1988). Ho et al. (1995), in our neighboring country Malaysia, however, did not report the presence of any fern spores in their study. Although Singapore is a highly urbanized society and ferns may generally only be found in fringes of the mangrove forests (*Stenochlaena palustris*) or as large patches of vegetation in open, abandoned areas or secondary forest (*Dicranopteris linearis* or *Dicranopteris curranii*), most of these ferns produce numerous, minute spores that can be dispersed widely by wind particularly during the drier periods. Some have, however, been used as ornamental plants (*Asplenium nidus*) owing to their beauty or grow on the numerous wayside trees within the urban environment. The inherent characteristics of spores, being smaller, lighter, and drier (less water component), also allow

them to remain airborne for a longer period of time, thus resulting in higher concentrations than pollen (Holttum 1968).

Allergy to fern spores has rarely been documented. This may have been owed to their low abundance in the temperate environment, coupled by the overwhelming presence of other allergenic sources, in particular, those of grass and tree pollen grains. However, sporadic reports of fern spore allergy have been reported. In Thailand, Bunnag et al. (1988) showed that more than 70% of the atopic patients tested showed skin reactions to extracts of fern spores and was further proven with nasal provocation. Positive skin reactions have been reported to spore extracts of ornamental ferns (Kanerva et al. 2001; Rodriguez et al. 2001; Kofler et al. 2000; Gellar-Bernstein et al. 1987). Also, contact allergy to ferns has also been reported in the literature (Stoof and Bruynzeel 1989; Hausen and Schulz 1978).

The widespread presence of ferns and their spores in our environment calls for a more detailed look at their importance as allergens. Spores of

Fig. 2 Fern spore calendar. Sums of the monthly fern spore counts are reflected as percentages of the year's total. The highest spore loads in concentrations (spores month⁻¹) are also indicated



Dicranopteris linearis and *Dicranopteris curranii* contain allergenic components that cause immediate hypersensitivity reactions (Chew et al. 2000). The specific IgE toward components of these spores has been detected in vitro.

In addition to the problem of allergenicity, the bracken fern (*Pteridium aquilinum*) has also been shown to be a health hazard by causing toxicity (Taylor 1990; Trotter 1990) and carcinogenic (Latorre et al. 2009; Smith and Seawright 1995). Spores of *Pteridium aquilinum* were noted in the Singapore airspora between 22.2 and 26.1% of the days surveyed (Table 4, Fig. 2). Coupled with pollutants in the urban setting, and notwithstanding its possible allergenicity, this spore type may pose an important health hazard to the population of Singapore.

The predominance of the pollen of oil palm or casuarina is unique as other reports from the region showed that pollen of the Poaceae are usually the most abundant (Ho et al. 1995; Phanichyakarn et al.

1989; Cua-Lim et al. 1978) (Table 2). Ho et al. (1995) nevertheless reported high concentrations of palm pollen in Malaysia, after those of the Poaceae, in their study. The exact species of palm however was not identified although they suggested that it may have been that of the royal palm (*Roystonea regia*) owing to the closeness of their sampling site to some of these palms. In this study, however, the oil palm pollen was detected in high concentrations at all three sites including the urban (Hougang) where the spore trap was placed on the roof top of a residential apartment block approximately 14 stories (45 m) above the ground, and where no known oil palm trees were found in the immediate vicinity.

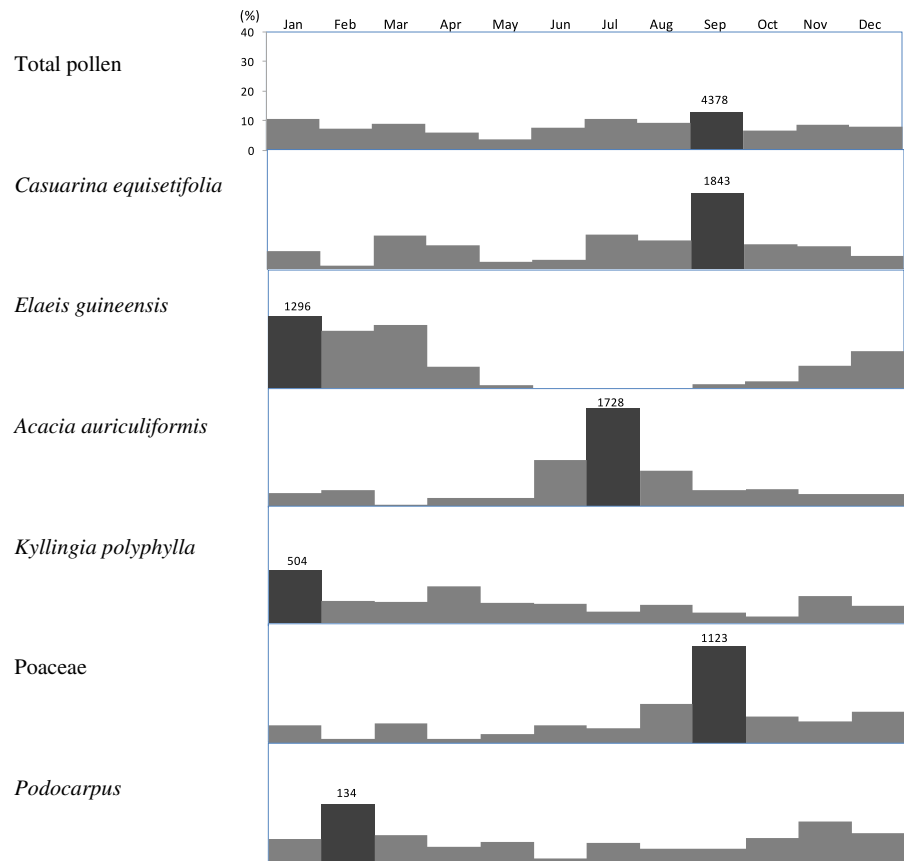
Though there are many grasses present in Singapore, grass pollen was not abundant in the air from 1991 to 1995 (Table 4). Initially, the sampling height was thought to be the contributing factor to this low number. Grasses are found at ground level hence only with strong wind conditions can grass pollen be

Table 5 Correlation of airspora through the studied years

Airspora type	1991			1992			1993			2005 and 2006		
	1992	1993	1994	1995	1993	1994	1995	1994	1995	2005	2006	1991
Total fern	+	++	+++			++				+++		++
<i>Nephrolepis auriculata</i>	+		+	+++		+	++	+++		+++		++
<i>Dicranopteris linearis</i>		+++	+++	+++	+	++				++	+++	+++
<i>Stenochlaena palustris</i>	+	+	+++	+++		+	+			+		+
<i>Asplenium nidus</i>												
<i>Pteridium aquilinum</i>			+			+						
<i>Dicranopteris curranii</i>			+									
Total pollen										+		
<i>Casuarina equisetifolia</i>				++			+	++				
<i>Elaeis guineensis</i>			+	+	+++	++	+++	+	++	+++		+
<i>Acacia auriculiformis</i>			+++	+++	+++	++			++	+++		++
<i>Kyllingia polyphylla</i>			+++	+++		++		++	++	++	+++	++
<i>Podocarpus</i>			+++	+++						++		
Poaceae												

Only significant correlations are shown for the top six fern or pollen types. Correlation p -values: + $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.001$. Spearman correlation was carried out for yearly airspora distribution. Averages from 2005 to 2006 were used in the analysis when they were found to be correlated for further analysis with airspora loads from 1991 to 1995. Counts from 2005 and 2006 were not correlated for *Asplenium nidus*, *Pteridium aquilinum*, *Dicranopteris curranii*, *Kyllingia polyphylla*, *Podocarpus*, and Poaceae; thus, each respective year was analyzed against those from 1991 to 1995. For 2005 analysis, only *Dicranopteris curranii* 1994 were correlated (+), and *Casuarina equisetifolia* 1991 (++) and Poaceae 1993 (+) were significantly correlated. No significant correlation was obtained for 2006 counts with those of 1991–1995 during analysis thus were not shown

Fig. 3 Pollen calendar. Sums of the monthly pollen counts are reflected as percentage of the year's total. The highest pollen loads in concentrations (pollen month⁻¹) are also indicated

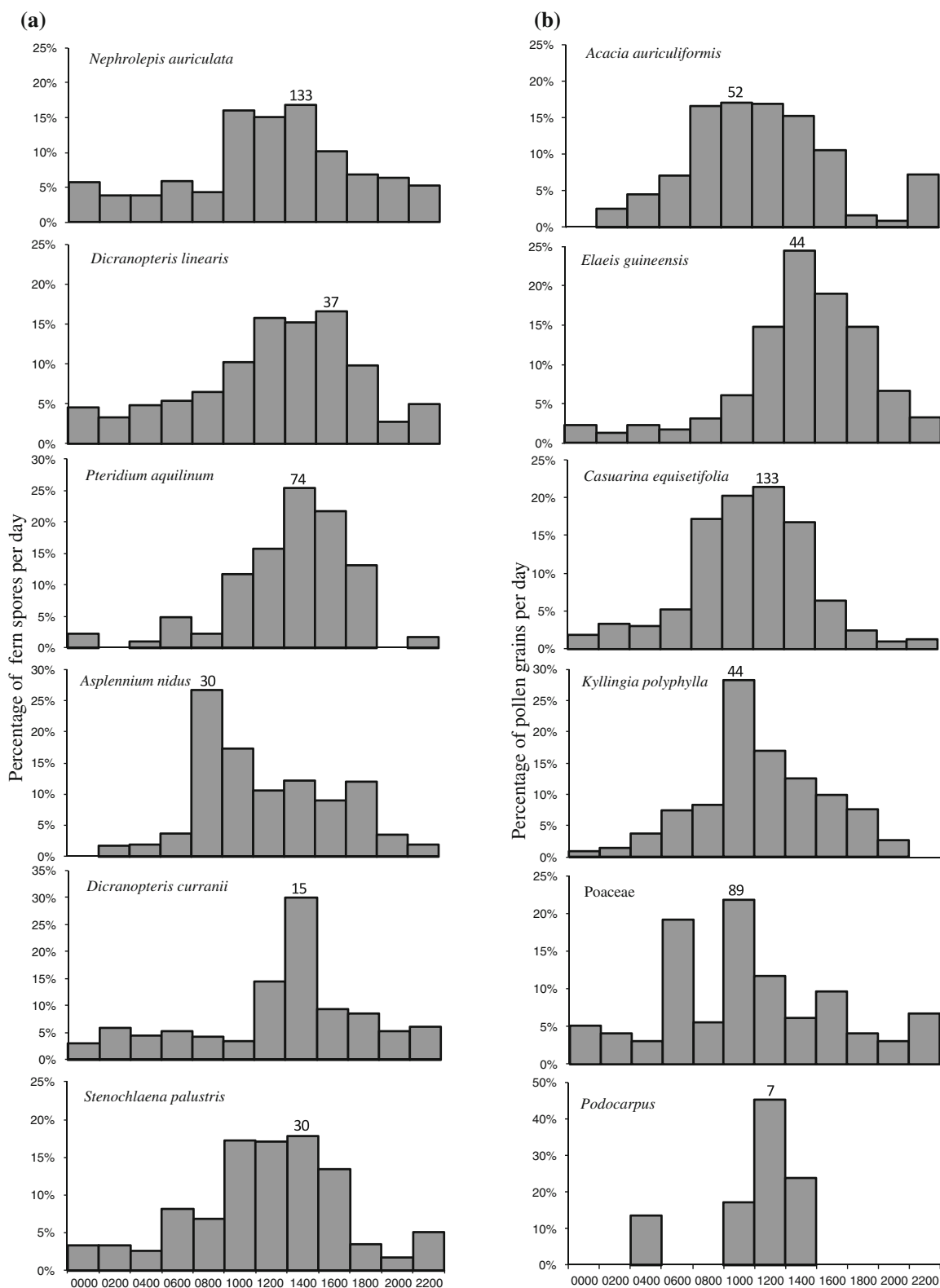


carried in the air and reach a height of 61 m above sea level, which is the height of the trap. A study conducted by Raynor et al. 1973 demonstrated that there was variation in the ragweed pollen concentration, with higher traps having a lower concentration than lower traps. However, this suggestion is dispelled by an increase in grass pollen being trapped at the same location in 2005–2006, resulting in grass pollen being ranked in the top three pollen contributors (Table 4). The initial low number of grass pollen being trapped is most probably owed to the lower presence of grasses in the vegetation. Grasses are regularly mowed in Singapore resulting in little or no opportunity to flower.

Very little, however, is known of the allergenicity of tropical pollen allergens although there has been a suggestive study (Chakraborty et al. 1996). A local preliminary screening of the degree of sensitization toward these airspora revealed that the local population was highly sensitized to their components (Chew et al. 2000). Oil palm pollen, in particular, was the

most allergenic, followed by the pollen of *Acacia auriculiformis*. Characterization of the immunochemical and allergenic components of the oil palm pollen revealed the presence of multiple allergens (Lee et al. 1995). Chakraborty et al. (1996) also demonstrated the allergenicity of palm pollen grains in Calcutta, India.

With regard to *Acacia*, reports from the southwestern regions of the USA showed that this predominantly ornamental taxon sheds sufficient airborne pollen to produce positive skin prick test reactions in patients suffering from pollinosis (Lewis and Vinay 1979). In Israel, *Acacia* is considered strongly allergenic (Lewis et al. 1983). *Acacia* bark and wood are reported to be impregnated with oils and terpenes and are considered a source of occupational allergens (Hausen and Schmalke 1981). Another report also showed that it may be an important occupational allergen in the Mediterranean region (Ariano et al. 1991). A report in Singapore has also demonstrated that immediate hypersensitivity to



◀ **Fig. 4** Diurnal patterns. **a** Fern spores and **b** pollen. The highest spores or pollen loads in concentrations (spores/pollen grains $\text{m}^{-3} \text{h}^{-1}$) are also indicated

Acacia pollen occurs in both humans and animals (Sin and Koh 1977). Whether the observed sensitization in the local population would translate into acute allergy symptoms is unknown. A Singapore study nevertheless indicated that the trends for acute asthma exacerbations (evaluated by asthma hospital admission figure and emergency room visits) were associated with the local airspora profile (Chew et al. 1998). Further evaluation and characterization of the allergenic components of our local airspora would be necessary to assess their role in allergic respiratory diseases in our local population.

Distinct changes were observed in the percentage contribution of the pollen types in airspora composition with an increase in unidentified pollen in 2005–2006 (Table 4). These changes occurred in tandem with Singapore's efforts to green the country through its Garden City initiatives (National Parks Board 2008). From 1986 to 2007, the green covering the country increased from 35.7 to 46.5%. Wayside trees were planted and parks were built to this effort. All these resulted in changes to the local vegetation with increased diversity of plants introduced to beautify the landscape, thus influencing the airspora composition. Variability in airspora trapped at different sites can also be attributed to the influence of the local vegetation. Changes in meteorological variables that affect plant growth and flowering will finally result in change in the plant composition of an area. Significantly higher average temperatures, minimum temperatures and solar radiation with lower maximum temperatures, humidities, and wind speeds were found in 2005–2006 when meteorological variables were compared to those of 1991–1995 (data not shown).

Fern spores and pollen are also present in our outdoor environment year-round although different spore or pollen types were found to be present in greater abundance at specific "seasons" (Figs. 2, 3). Studies elsewhere in Southeast Asia support this observation of the presence of seasonal trends in atmospheric airspora concentration. In Malaysia, pollen and spore counts appear to peak in March and have a minor peak around September (Ho et al. 1995). Similarly, pollen concentrations were found to peak November–February in Bangkok, Thailand

(Phanichyakarn et al. 1989), while in the Philippines, the highest pollen counts were obtained November–January and lowest in June (Cua–Lim et al. 1978). As in temperate countries, a better understanding of the seasonal distribution of possible allergenic airspora and the factors that would influence their atmospheric concentrations would be important to alert allergy sufferers and facilitate effective management of pollen specific allergies.

Airspora diurnal patterns displayed by plants are circadian rhythms probably controlled through their genetic makeup and their relationships with environmental factors. A very good example of this biological program can be seen clearly in the study by Reddi et al. (1988). By studying anther dehiscence of 54 grass species in Visakhapatnam, India, during the height of the flowering season of each, the grass release patterns were categorized into 10 types: 24-h, bimodal frequency, pre-middle night, middle-night, post-middle night, early morning, forenoon, middle-day, afternoon, and evening. A uniform environmental condition was present to all these grass species that displayed unique circadian rhythms.

Generally, factors affecting the production, release, and transport of fern spores or pollen can affect the kinds and concentration of them present in the air (Gioulekas et al. 2004). One of the important factors governing them is meteorology. Factors such as temperature, relative humidity, and rainfall can affect the development of the plants and hence the production of fern spores or pollen. Temperature and relative humidity can dehydrate the anthers and cause them to burst and release the pollen into the air (Solomon 1984). Since spores are also contained within sporangia, increasing temperature and low humidity can similarly cause the drying of the sporangium walls, causing them to open and release the spores. Also, rainfall, wind speed, and direction can affect the dispersal and transport of airspora. All these in turn affect the presence or absence, and concentration of spores or pollen grain in a particular area.

Small differences in the daily temperature range as seen by the positive correlation with minimum temperature and negative correlation with maximum temperature were shown to promote dispersal of the major fern spores and pollen (Table 6). The temperature gradient has a pronounced effect on sea breezes because owing to the difference in temperature of the

Table 6 Concentration of airspora and meteorological factors

	T _{mean} (°C)	T _{max} (°C)	T _{min} (°C)	RF (mm)	RH ₁₅₀₀ (%)	RH ₀₉₀₀ (%)	TSR (W m ⁻²)	TWS (m s ⁻¹)
Total fern	0.294***	-0.145***	0.291***	-0.131***	-0.269***	-0.197***	0.231***	-0.246***
<i>Nephrolepis auriculata</i>	0.27***	-0.167***	0.285***	-0.068***	-0.194***	-0.133***	0.227***	-0.208***
<i>Dicranopteris linearis</i>	0.145***	0.079***	0.072***	-0.213***	-0.233***	-0.154***	0.122***	-0.06**
<i>Stenochlaena palustris</i>	-0.044*	0.045*	-0.074***	ns	ns	ns	-0.042*	ns
<i>Asplenium nidus</i>	-0.108***	ns	-0.123***	0.119***	0.047*	0.091***	-0.088***	ns
<i>Pteridium aquilinum</i>	-0.043*	0.115***	-0.11***	ns	ns	-0.049*	-0.107***	-0.057**
<i>Dicranopteris curranii</i>	0.158***	ns	0.143***	-0.126***	-0.184***	-0.111***	0.136***	ns
Total pollen	0.217***	-0.103***	0.237***	-0.122***	-0.301***	-0.252***	0.193***	-0.054**
<i>Casuarina equisetifolia</i>	0.117***	ns	0.113***	-0.055**	-0.143***	-0.149***	0.087***	-0.145***
<i>Elaeis guineensis</i>	-0.079***	-0.091***	-0.052*	ns	-0.15***	ns	ns	0.282***
<i>Acacia auriculiformis</i>	ns	0.139***	ns	-0.145***	ns	-0.045*	ns	0.068***
<i>Kyllingia polyphylla</i>	0.273***	-0.107***	0.294***	-0.085***	-0.27***	-0.134***	0.203***	-0.048*
<i>Podocarpus</i>	-0.087***	ns	-0.094***	-0.043*	ns	ns	ns	0.147***
Poaceae	0.064**	ns	0.066**	ns	-0.091***	-0.085***	ns	-0.065**

Only the top six fern or pollen types are shown ($n > 1,822$ days). Data from the Kent Ridge station collected from 1991 to 1995 and 2005–2006 were used in this analysis. Meteorological parameters: T_{mean} average daily temperature, T_{max} maximum daily temperature (°C), T_{min} minimum daily temperature, RH_{0900} relative humidity at 0900 h, RH_{1500} relative humidity at 1500 h, RF total daily rainfall, TWS total wind speed, and TSR total solar radiation. Spearman correlation coefficients; p -values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

ns not significant

Table 7 Diurnal concentration of airspora and meteorological factors

	Temperature (°C)	Rainfall (mm)	Relative humidity (%)	Total wind speed (m s ⁻¹)	Total solar radiation (W m ⁻²)
<i>Nephrolepis auriculata</i>	0.208***	−0.045*	−0.139***	ns	0.188***
<i>Dicranopteris linearis</i>	0.153***	ns	−0.144***	ns	0.137***
<i>Stenochlaena palustris</i>	0.064**	ns	−0.071**	0.043*	0.072***
<i>Asplenium nidus</i>	ns	ns	ns	ns	0.068**
<i>Pteridium aquilinum</i>	0.062**	ns	−0.045*	0.051*	0.077***
<i>Dicranopteris curranii</i>	0.119***	ns	−0.126***	ns	0.095***
<i>Casuarina equisetifolia</i>	0.125***	ns	−0.086***	0.072***	0.159***
<i>Elaeis guineensis</i>	0.163***	−0.044*	−0.245***	0.257***	0.185***
<i>Acacia auriculiformis</i>	0.108***	ns	−0.052*	ns	0.084***
<i>Kyllingia polyphylla</i>	0.113***	ns	−0.084***	0.085***	0.15***
<i>Podocarpus</i>	0.052*	ns	−0.049*	0.046*	0.07**
Poaceae	ns	ns	−0.052*	ns	ns

Only significant correlations of the top six fern or pollen types are shown ($n > 177$ days). Spearman correlation coefficients; p -values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

ns not significant

island land mass and surrounding water, sea breezes are common in Singapore (National Environmental Agency 2007). Sea breeze causes spores and pollen to be dispersed and brings humidity inland, frequently resulting in rain in the afternoons. Rains wash out the airborne particles resulting in lower densities of spores and pollen (Davies 1974).

A smaller daily temperature range will reduce the difference in temperature between land and sea, thus resulting in a reduced sea breeze. Relative humidity and wind speed are components affected by the sea breeze effect. These same factors are also variables found to be often correlated with airspora levels for daily and diurnal patterns. Furthermore, factors such as total solar radiation influence plant growth.

For oil palm pollen densities, a positive correlation was observed for wind speed. This can be owed to the source of the pollen itself (Tables 6, 7). Oil palm is not commonly cultivated in Singapore but is a major crop in neighboring Malaysia, and very likely, pollen is transported in by the wind. The occurrence of high pollen densities during the northeast monsoon seasons from December–March and the late diurnal peak hour compared to other pollen types suggests the involvement of distant transportation.

It is thus evident that allergenic or toxic spores and pollen are present in the outdoor atmosphere of Singapore. Previous studies have shown that the use

of currently available commercial allergenic extracts for the evaluation of immediate hypersensitivity in the local population, particularly to environmental pollen and spores, has not been very indicative or of any diagnostic value (Tan and Teoh 1979; Lim et al. 1983; John et al. 1996). The need for a local panel of allergens for the diagnosis of inhalant allergies is thus highlighted. Additionally, further review and characterization of the allergens important to the local context are needed. This survey of the airborne spores and pollen has thus provided the baseline data for local environmental triggers of allergy in Singapore and the region.

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