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Outdoor airborne fungal spores in Singapore

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A continuous sampling of airspora in Singapore was carried out over a period of 5 years at 3 different sites. Fungal spores were found to be numerically dominant, comprising between 86.0–89.4% of the total airspora which also consisted of spores of fern and pollen grains. Conidia of *Cladosporium* were the most abundant fungal spore type, followed by the ascospores of *Didymosphaeria*, and the conidia of *Curvularia*, *Drechslera* and *Pithomyces*. Variations in fungal spore counts were discernible. Two periods of high spore densities were observed annually; in the months of February–March, and October–November. Minor peak periods were observed occasionally in April and July–August. The seasonal trends of the 5 most common fungi generally coincided with these same peak periods for spore counts. Correlation analysis showed that fungal spore counts were influenced by meteorological factors, especially temperature and relative humidity. In particular, *Didymosphaeria* ascospore counts were positively correlated with relative humidity and total daily rainfall, and inversely correlated with temperature and wind speed. Conversely, *Curvularia*, *Drechslera* and *Pithomyces* spore counts decreased with increases in relative humidity and precipitation. The spore counts of *Curvularia* and *Cladosporium* were also positively correlated with temperature, while those of *Drechslera* were positively correlated with wind speed. This study revealed the existence of a rich fungal airspora in Singapore.

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The study of airborne particles, including fungal spores, is important to mycologists, plant pathologists, microbiologists, as well as allergists. The fungal content of airspora has been examined in many parts of the world (Cosentino et al. 1990, Shaheen 1992, Halwagy 1994). By comparison, the number of aerobiological surveys in tropical South-East Asia is limited. Only a few studies have been carried out, namely in Thailand (Tuchinda et al. 1983, Phanichyakarn et al. 1989) and Malaysia (Ho et al. 1995). A preliminary study in Singapore has demonstrated the presence of a multitude of fungal spores in the outdoor environment (Tan et al. 1992).

In view of the paucity of aerobiological data in the tropics, particularly in South East Asia, an aerobiological survey of the Singapore environment was undertaken. In this paper, we report on the results of a 5-year study (June 1990 – June 1995) on the outdoor airborne fungal spores in Singapore.

MATERIALS AND METHODS

Continuous volumetric air sampling was carried out with Burkard seven day volumetric recording spore traps (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK) on the rooftops at three locations in Singapore (Figure 1).

(1) Kent Ridge Station – Located at the Department of Biological Sciences, National University of Singapore, this station was situated approximately 61 metres above ground level at the edge of a secondary forest.

(2) Clementi station – Located at Clementi Town Secondary School, this station was sited approximately 28 metres above ground level in an urban township.

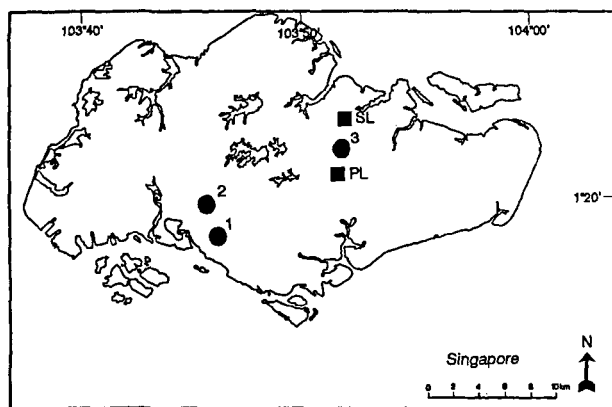


Fig. 1. Map of Singapore with the locations of the sampling sites and meteorological stations. (1) Department of Biological Sciences, Block S3, National University of Singapore, Lower Kent Ridge Road (Kent Ridge station); (2) Clementi Town Secondary School, Science Block, Clementi Avenue 3 (Clementi station); (3) Block 25, Housing and Development Board flat, Hougang Avenue 3 (Hougang station). Meteorological data were recorded at Sites (1) and (2) while data for site (3) were obtained from Seletar (SL) and Payar Lebar (PL) meteorological stations.

(3) Hougang station – Located at a public housing residential apartment block, this station was situated approximately 45 metres above ground level in an urban township.

The periods of the survey were from June 1990 to June 1995 for the Kent Ridge and Clementi stations, and from June 1993 to June

1995 for the Hougang station. Meteorological data were simultaneously recorded on site at Kent Ridge and Clementi using an Envirodata Automatic Weather Station (Queensland, Australia). The parameters measured included precipitation (daily total and duration) and evaporation rate, wind speed and direction, temperature, relative humidity, atmospheric pressure and solar radiation. Meteorological data for the Hougang station were obtained from meteorological stations at Seletar (SL) and Paya Lebar (PL) (see Figure 1).

The traps were operated continuously at a flow rate of 10 litres min⁻¹. Airborne particles were trapped on a Melinex tape coated with an adhesive (vacuum grease silicone, Beckman Instruments Inc., catalog no. 335 148). The tape was mounted on a rotating drum driven by clockwork at a rate of 2 mm hour⁻¹ and changed weekly. The sampling and quantification methods used in this study were similar to standardized procedures described by Hirst (1952) and as recommended by the manufacturer. Briefly, the used tape was cut into segments representing each day of the week, and mounted on glass slides. Counts were made by light microscopy at 25× objective (10× eyepiece) with a microscopic field diameter of 0.71 mm. Daily counts (7 days a week) of all spore and pollen categories were estimated by counting the entire traverse stripe at five points of the width of the segment, the stripe being parallel to the direction in which the segment moved. In addition, the entire segment was also screened for spores and pollen that were not represented on the traverse stripe. For these spores or pollen, the entire segment was screened to obtain a spore count.

Daily spore counts were converted to the number of spores m⁻³ per day, and the frequency of occurrence, spore counts (spores m⁻³ per day) (mean and maximum values), and annual and monthly variations presented. Statistical analysis was done by non-parametric methods. Correlation studies between spore counts and meteorological factors were performed via the Spearman's rank correlation test using statistical package SAS v6.08 for windows.

RESULTS

Airspora components

The 5-year aerobiological survey revealed the existence of a rich airspora in the atmosphere at the three sites sampled. The major components of the airspora of the 5-year survey for Kent Ridge and Clementi stations, and the 2-year survey for Hougang are listed in Table I. At all three sites, fungal spores made up 86–89.4% of the total airspora, followed by 0.2–8.6% fern spores and 4.4–5.4% of pollen grains.

Among the fungal components, spores of the Deuteromycetes were predominant, with *Cladosporium* being the most abundant (33.5–41.0% of the total airspora). *Cladosporium* spores were also found between 65.6–69.5% of the total number of days surveyed (Table II). The next most abundant fungal spore was *Didymosphaeria* (21.9–28.6% of total airspora), followed by the conidia of *Pithomyces* (10.2–14.7%), *Curvularia* (4.1–10.6%) and *Drechslera* (1.4–2.3%). Other fungal spore types which contributed less than 1% of the total airspora counts included *Alternaria*,

Beltrania, *Didymopleela*, *Grallomyces*, *Hiospira*, *Pleospora*, *Pringsheimia*, smut fungus, *Tetraploa* and *Torula*. Other genera of fungi which were trapped sporadically and at very low frequency (<1% of the total number of days surveyed) included *Cercosporidium*, *Dictyoarthrinium*, *Leptosphaeria*, *Passeriniella*, *Spegazzinia*, and *Stigmina*. Less than 1% of the airspora could not be identified and were listed as 'unknown fungi', which included small colourless spores and unidentified basidiospores.

Correlation of spore counts between sampling sites

The total fungal spore counts of all 3 sites were significantly correlated (Table III). In terms of individual spore types, significant correlation was observed between spore counts of *Cladosporium*, *Curvularia*, *Didymopleela*, *Didymosphaeria*, *Drechslera*, *Grallomyces*, *Pithomyces*, *Tetraploa* and *Torula* from all 3 sites. Other spore types, in particular those with lower abundance did not correlate between sites. Differences in local vegetation and microclimatic factors, which can influence the proliferation and subsequent dispersal of fungal spores, are likely reasons for the poorer, or lack of correlation of these spores between stations. Additionally, this could also be due to a statistical problem. As counts for these spores are low and as such their coefficients of variability tended to be high, the correlations would thus be weak or not significant at all.

It was also noted that fungal spore counts at Kent Ridge and Clementi, situated near each other in the south-western region of Singapore, were better correlated with each other than with the counts at Hougang, a site situated in the north-eastern part of the island.

Annual variation

The annual contribution of each spore type to the total airspora counts varied slightly from year to year. The fluctuation in the relative abundance of *Cladosporium*, *Curvularia*, *Didymosphaeria*, *Drechslera* and *Pithomyces* are shown in Table IV. The absolute numbers of spores of the 5 common fungi varied from year to year.

Monthly variation

Fig. 2 illustrates the monthly variation for each spore type and the total fungal spore count (average of 5 years). Only spore types which contributed more than 1% to the total airspora counts are presented. The monthly profile of the total fungal airspora did not differ significantly among sites. Fungal spores were found in the atmosphere perennially (24 hours geometric mean [average of 3 sites] = 1 688 spores m⁻³ day⁻¹) but distinct monthly variation in the spore densities were observed (maximum = 19,075 spores m⁻³ day⁻¹). In general, fungal spore counts usually peaked in February–March, and October–November. Minor peak periods were observed occasionally in April and July–August.

There were 4 peak periods of high spore counts for *Cladosporium* (> 700 spores m⁻³ day⁻¹; geometric mean = 738 spores m⁻³ day⁻¹) at Kent Ridge and Clementi; beginning with a rather broad period of high spore counts from February–

Table I. Major components (% total airspora) of the airspora in Singapore.

Airspora component	Kent Ridge	Clementi	Hougang
Fungal spores	88.1	89.4	86.0
Fern spores	6.6	6.2	8.6
Pollen	5.3	4.4	5.4

Table II. Percentage contribution of fungal spore types to the total airspora count* and percentage number of days of spore types occurring over the total number of days surveyed **.

*Total airspora count (including fungal spores, fern spores and pollen) for the entire survey period: Kent Ridge=3 254 521; Clementi=4 106 218; Hougang=1 514 937. **Total number of days surveyed: Kent Ridge=1 822; Clementi=1 788; Hougang=729. † Only spore types which occurred in more than 5% of the total number of days surveyed or more than 1% of total spore counts are listed. Spore types are listed in descending order of their contribution to the total counts.

Spore type†	Kent Ridge		Clementi		Hougang	
	% total spores	% days occurred	% total spores	% days occurred	% total spores	% days occurred
<i>Cladosporium</i>	41.0	65.6	33.5	69.5	38.2	66.6
<i>Didymosphaeria</i>	27.5	88.9	28.6	90.4	21.9	84.8
<i>Pithomyces</i>	11.0	91.9	14.7	93.5	10.2	93.5
<i>Curvularia</i>	4.1	84.7	7.3	90.6	10.6	95.1
<i>Drechslera</i>	1.4	65.4	1.4	68.0	2.3	80.7
Smut fungus	0.7	15.2	0.9	21.7	0.5	12.0
<i>Tetraploa</i>	0.4	56.0	0.4	68.1	0.7	79.6
<i>Didymopleela</i>	0.3	23.7	0.3	24.3	0.5	28.0
<i>Pleospora</i>	0.3	23.3	0.4	29.3	0.5	27.2
<i>Pringsheimia</i>	0.2	11.8	0.2	13.6	0.1	7.9
<i>Torula</i>	0.2	22.5	0.3	26.0	0.4	38.2
<i>Alternaria</i>	0.1	13.0	0.1	12.0	0.0	6.7
<i>Hiospira</i>	0.1	14.0	0.0	15.7	0.2	19.7
<i>Beltrania</i>	0.1	9.9	0.1	10.7	0.1	5.0
<i>Grallomyces</i>	0.0	9.8	0.0	10.1	0.1	12.2
Unknown fungi	0.7	47.5	0.6	48.9	0.9	46.6

Table III. Correlation between fungal spore counts at Kent Ridge, Clementi and Hougang. Spearman's correlation coefficients (p-value‡).

†Only spore types which occurred in more than 5% of the total number of days surveyed or more than 1% of total spore counts are listed. Spore types are listed in descending order of their contribution to the total spore counts. ‡Correlation p-values: *p<0.05; **p<0.01; ***p<0.001; Correlation coefficients which were not significant are not listed.

Spore type†	Kent Ridge vs Clementi	Clementi vs Hougang	Hougang vs Kent Ridge
Total fungi	0.31***	0.26***	0.15***
<i>Cladosporium</i>	0.44***	0.22***	0.16***
<i>Didymosphaeria</i>	0.47***	0.36***	0.20***
<i>Pithomyces</i>	0.24***	0.16***	0.10*
<i>Curvularia</i>	0.28***	0.36***	0.23***
<i>Drechslera</i>	0.15***	0.17***	0.10**
Smut fungus	0.14***		0.10*
<i>Tetraploa</i>	0.15***	0.14**	0.14**
<i>Didymopleela</i>	0.19***	0.16***	0.24***
<i>Pleospora</i>			
<i>Pringsheimia</i>	0.10***		
<i>Torula</i>	0.13***	0.17***	0.09*
<i>Alternaria</i>	0.08**		
<i>Hiospira</i>	0.07**		
<i>Beltrania</i>	0.06*		
<i>Grallomyces</i>	0.23***	0.16***	0.13**

April, June–July, September and a major peak from October–November (Figure 2). At Hougang, the peaks during January–February and October–November were more distinct.

With spores of *Curvularia*, the monthly trends were observed to be similar for Clementi and Hougang except for the first quarter (Figure 2). There was a peak period of high

spore counts in February–March at Clementi whereas Hougang showed a gradually increasing trend in the first quarter of the year with a small peak in April. Three other periods of high spore counts were seen in June, August and October. Monthly variation of *Curvularia* spores was not apparent at Kent Ridge where these spores occurred constantly throughout the year, and in counts lower than the 2 urban sites (geometric mean=67 spores m⁻³ day⁻¹ at the edge of secondary forest; 157 spores m⁻³ day⁻¹ in the urban setting).

The monthly pattern for *Didymosphaeria* was very similar at all three sites (Figure 2). Two major periods of high spore counts were observed (geometric mean=489 spores m⁻³ day⁻¹; maximums=9,926 spores m⁻³ day⁻¹). There was a broad peak from February to April and a second in November. With *Drechslera*, however, spore counts remained relatively constant throughout the year (25–46 spores m⁻³ day⁻¹) except for the beginning or the end of the year (January to March, and November to December) when the counts showed an increase.

Three periods of high spore counts were recorded for *Pithomyces* at Clementi, but not at the other two sites (Figure 2). The peaks occurred in February, July and October. Spore counts were generally higher in Clementi (geometric mean=289 spores m⁻³ day⁻¹) than in Kent Ridge and Hougang where less marked fluctuations were observed (geometric mean=190 spores m⁻³ day⁻¹).

Association with meteorological variables

Correlations between spore counts of the five most abundant fungal species and meteorological variables at the three survey sites are shown in Table V. Total fungal spore counts were observed to have a positive correlation with relative

Table IV. Annual percentage contribution of fungal spore type† to the total spore count*.

*Total number of airspora includes fungal spores, fern spores and pollen. (†) Only spore types which occurred in more than 5% of the total number of days surveyed or more than 1% of total spore counts are listed. Spore types are listed in descending order of their contribution to the total spore counts.

Spore type	Kent Ridge						Clementi						Hougang		
	'90	'91	'92	'93	'94	'95	'90	'91	'92	'93	'94	'95	'93	'94	'95
<i>Cladosporium</i>	60.6	57.8	22.7	23.0	37.5	31.1	55.2	50.8	20.6	21.8	27.5	23.2	26.2	44.4	31.7
<i>Didymosphaeria</i>	15.2	19.8	41.5	35.8	26.8	32.0	19.9	21.9	37.1	33.4	27.5	31.9	27.4	20.6	19.5
<i>Pithomyces</i>	4.8	7.1	14.5	16.5	12.8	13.4	5.5	8.6	18.0	20.2	17.8	18.6	11.5	9.1	12.0
<i>Curvularia</i>	2.1	2.9	4.2	4.8	6.0	6.0	3.0	6.1	7.5	6.8	11.1	8.4	11.3	9.8	12.8
<i>Drechslera</i>	1.4	1.0	1.4	1.6	1.9	1.8	1.5	1.1	1.4	1.1	1.5	1.7	2.3	2.2	2.8
<i>Smut fungus</i>	0.7	0.6	1.1	0.6	0.6	0.5	0.8	1.1	1.8	0.5	0.6	0.5	1.2	0.4	0.1
<i>Tetraploa</i>	0.3	0.2	0.5	0.5	0.4	0.4	0.3	0.4	0.4	0.4	0.5	0.5	0.8	0.5	1.1
<i>Didymopleela</i>	0.2	0.2	0.3	0.3	0.3	0.4	0.8	0.2	0.3	0.4	0.3	0.3	0.6	0.4	0.4
<i>Pleospora</i>	0.3	0.2	0.3	0.5	0.4	0.4	0.4	0.2	0.3	0.5	0.5	0.4	0.7	0.5	0.3
<i>Pringsheimia</i>	0.2	0.1	0.1	0.3	0.2	0.5	0.1	0.2	0.1	0.1	0.2	0.3	0.1	0.1	0.3
<i>Torula</i>	0.2	0.2	0.2	0.3	0.2	0.4	0.2	0.2	0.2	0.3	0.3	0.6	0.5	0.3	0.6
<i>Alternaria</i>	0.1	0.1	0.1	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0
<i>Hiospira</i>	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.3	0.1	0.1
<i>Beltrania</i>	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1
<i>Gallomyces</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
Unknown fungi	0.8	0.6	0.6	0.8	0.5	0.4	0.7	0.6	0.7	0.8	0.5	0.5	1.6	0.8	0.8

humidity at all sites. The spore counts of *Didymosphaeria*, an ascomycete, correlated positively with relative humidity and total rainfall (mm day^{-1}), and inversely with temperature and wind speed. In contrast, spore counts of *Cladosporium*, *Curvularia*, *Drechslera*, *Pithomyces* correlated negatively with relative humidity and total rainfall, and positively with temperature and total wind speed.

DISCUSSION AND CONCLUSIONS

The predominance of *Cladosporium* spores in this study was not surprising as *Cladosporium* is ubiquitous and its dominance in other parts of the world has been well documented (Cosentino et al. 1990, Shaheen 1992, Dames & Cadman 1994, Halwagy 1994). *Didymosphaeria* was the only genus in the Ascomycetes which was found in abundance in this survey. This is unique to the present study as it has not been reported in airspora elsewhere. Of interest, *Pithomyces* was third in abundance (10.2–14.7%) in Singapore whereas it was present at less than 1% in Thailand (Phanichyakarn et al. 1989), Durban, South Africa (Dames & Cadman 1994), Kuwait (Halwagy 1994), Madras, India (Vittal & Krishnamoorthi 1989) and Dehra Dun, India (Singh et al. 1987). The occurrence of *Curvularia* was comparable to surveys conducted in India (Kumar 1982, Singh et al. 1987, Vittal & Krishnamoorthi 1989) and Malaysia (Ho et al. 1995). In a preliminary outdoor airspora study in Singapore however, *Nigrospora sphaerica* instead of *Cladosporium* and *Didymosphaeria* was found to be the major component (Tan et al. 1992). This may have been due to the different methods used to trap fungal spores in these surveys. In the earlier study, a Biotest Centrifugal Air Sampler was used to sample the airspora (as oppose to a continuous Burkard seven day volumetric spore traps used in this study). The former required the spores trapped on rose bengal agar strips to be cultured, identified and quantified based on colony forming

units, and thus may have been selective for certain genera of fungi.

In this study, some differences were observed between the spore profiles at different sites (see Figure 2). Differences in spore counts at different sites have been reported by Hamilton (1959) and Lacey (1962). The latter was of the opinion that "local ecology was important in determining both the concentration and the composition of airspora in the atmosphere at each site." In the present study, the total fungal spore count was generally lower at Kent Ridge than at Clementi and Hougang. It is possible that these differences were attributed to variations in environmental conditions such as density and type of vegetation, human activities which can influence the number and kinds of fungi present, as well as the ecology and behaviour of the fungi present at each site.

Despite the absence in the tropics of the four climatic seasons that characterise temperate countries, monthly variations in spore counts were observed. Such variations have also been reported in a number of studies conducted in the tropics. The monthly profile for total fungal airspora was quite similar to that reported by Singh et al. (1987) in Dehra Dun, India, where a broad peak period was observed from February to April and at the year-end from September to November. In Thailand, however, peak counts of fungal spores were observed in December through February with slightly lower counts in November, April and May (Phanichyakarn et al. 1989). In Malaysia, Ho et al. (1995) found 2 peaks for total fungal spore counts. The first peak was in February and the second in October.

Ho et al. (1995) observed that the total fungal spore counts followed closely that of *Cladosporium*. The airspora profile for *Cladosporium* in Singapore also peaked in the months of January–February and October–November (distinctly observed at Hougang). This profile differed from other reports from India (Vittal & Krishnamoorthi 1989) and Thailand (Bunnag et al. 1982) which noted consistently

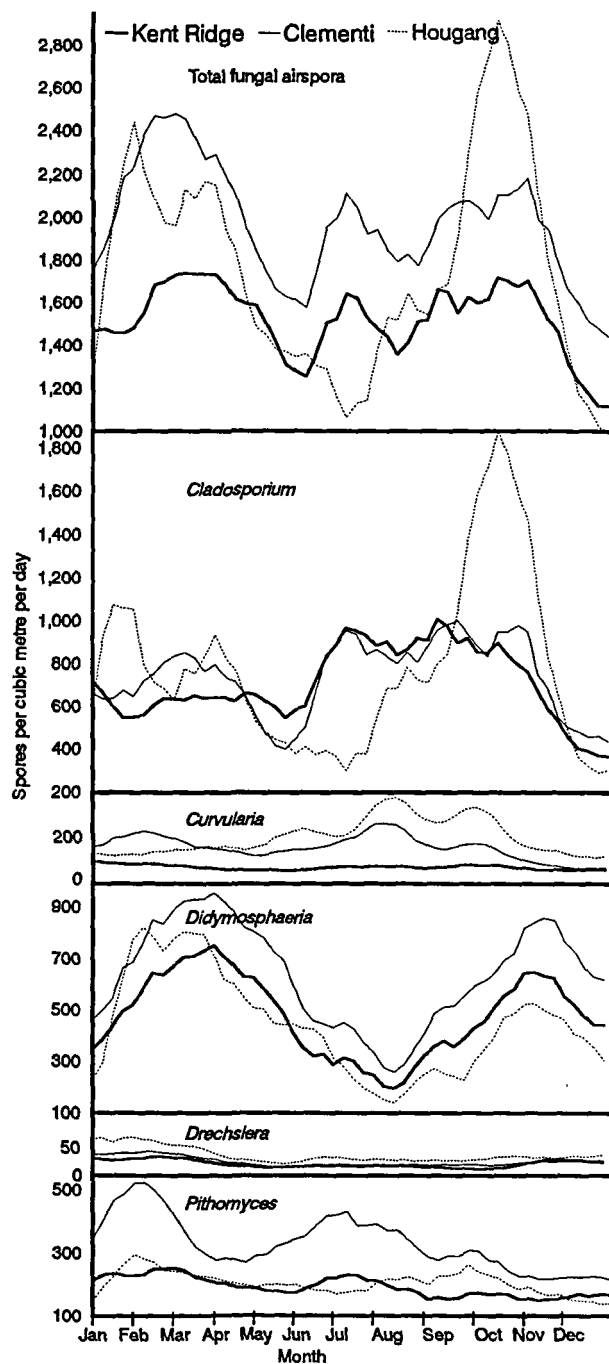


Fig. 2. Monthly variations in fungal spore counts in Singapore, 1990–1995. Only the top five fungal spore types are shown.

high numbers of *Cladosporium* from December to February. In temperate climates, Flensburg and Samsøe-Jensen (1950) observed a monthly peak for *Cladosporium* from June to mid October (summer–autumn) in their 2-year survey of the atmosphere in Copenhagen (Denmark). Cosentino et al. (1990) observed high counts of *Cladosporium* in May–June (spring) and September–November (autumn) while the concentration decreased in winter and summer (July–August) in Italy. The release of *Cladosporium* spores is facilitated by

dry, windy conditions since it is considered a member of the “dry airspora”. This observation is supported by the studies of Palmas and Cosentino (1990) who found an inverse relationship between relative humidity and *Cladosporium* spore counts in Sardinia, Italy; and of Ho et al. (1995) who reported that high counts of this fungus in Malaysia were recorded together with low rainfall and relative humidity, and high day temperatures.

In this study, there was a weak but significant inverse correlation between precipitation and *Cladosporium* at Kent Ridge. Such an inverse correlation had been reported by Hyde and Williams (1953), Palmas and Cosentino (1990) and Hjelmroos (1993). However, other workers have observed a direct relationship between counts of *Cladosporium* and rainfall. Richards (1956) observed that an absence of rainfall reduced the numbers of *Cladosporium* spores in the air, while Hirst (1953) noted a transient increase in *Cladosporium* at the start of rain. It has also been reported by Rantio-Lehtimäki (1977) that the rainy summer of 1977 in Finland showed the highest spore concentration for the 4-year sampling period with *Cladosporium* contributing to the high peak counts. These contradicting reports may be explained by Rich and Waggoner's (1962) suggestion that rain had two effects on the concentration of airborne *Cladosporium* spores: gentle rains washed spores down, gusty rainstorms carried more spores aloft into the atmosphere.

Interestingly, we found high counts (21.9–28.6% of total airspora) of *Didymosphaeria* (an Ascomycete) the year round. High frequency of ascospores was also observed by Shaheen (1992) in Amman, Jordan but only on days of high humidity. Hirst (1953) reported brief increases of ascospores in the air after rain, during periods of high humidity and in the nights in summer and autumn when the air was damp. Royes (1987) observed high total counts of Ascomycetes, together with Fungi Imperfecti and Basidiomycetes, during months with the highest rainfall. These observations suggest that the dispersal of ascospores may be aided by the mechanical effect of rain.

In this study, *Curvularia* was found to peak in February, June, August and October. Bunnag et al. (1982) also observed a peak for *Curvularia* in October in Thailand but in Malaysia (Ho et al. 1995), *Curvularia* appeared to peak in January, June and September. In correlation studies with meteorological parameters, *Curvularia* showed an inverse correlation with rainfall and relative humidity but a direct correlation with temperature. The lower counts for *Curvularia* during dryer months may be explained by the mechanism by which *Curvularia* discharges its conidia. According to Meredith (1963), as the basal cell in each conidium loses water by evaporation, it decreases in volume. However, when a gas phase appears, the basal cell tends to spring back to its original shape. The gas phase provides the jolt to break the conidial attachment as the basal cell springs back to its original shape and the conidium springs away.

Although wind is thought to be important in moving spore clouds, the relationship between wind and counts of fungal spores in the air is variable, as can be seen from various reports. While Turner (1966) and Frey and Durie (1960) found no correlation in the incidence of atmospheric spores with either changes in wind velocity or direction, Lyon et al.

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