



Short communication

A fungal spore calendar for the atmosphere of Melbourne, Australia, for the year 1993

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Abstract

A calendar of fungal spore seasons for Melbourne during 1993 was established using a 7-day volumetric Burkard trap. Twenty-nine genera and five spore groups were identified. The dominant spore types detected were *Cladosporium* (41.7%), *Leptosphaeria* (14.9%), *Coprinus* (14.6%), 'Ascospore 1' (5.5%), *Ganoderma* (2.1%) and *Alternaria* (1.4%). Seasonally, spore levels of *Cladosporium* and *Alternaria* peaked in spring and summer, *Leptosphaeria* and *Ganoderma* peaked toward summer and autumn, 'Ascospore 1' peaked in winter, whilst spore levels of the basidiomycete *Coprinus* fluctuated year round. In conclusion, a range of allergenic fungal spores were present in the air of Melbourne throughout the year.

Introduction

The seasonal distribution of fungal spores in the atmosphere has important health implications for allergy and asthma sufferers. Given that a number of fungal genera are allergenic (e.g. *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus*), the characterisation of fungal spore seasons can allow physicians and allergy sufferers to more accurately establish preventative strategies. In previous work (Mitakakis et al., 1997) we examined the annual and seasonal variations between the years 1992 to 1994, segregating spores of *Cladosporium* and *Alternaria* from total spore numbers. There has not yet been a quantitative investigation of the range of airborne spores in Melbourne's atmosphere. Thus a daily spore calendar has been established for Melbourne, Australia, studying the year 1993 in detail, using a Burkard trap.

Materials and methods

Daily spore counts

Daily spore counts were collected between January and December, 1993 using a Burkard 7-day volumetric trap (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, U.K.). Spores were trapped onto adhesive tape (10% Dow Corning A280 series in xylene on melinex tape), then cut into daily segments. The segments were stained with Calberla's stain for pollen identification, then viewed by light microscopy at a magnification of 500× for fungal spores along a full lengthwise traverse. The spore concentrations were estimated as the number of spores per cubic metre of air sampled, as according to Kapyla and Penttinen (1981).

Location of Burkard spore trap

The air sampler was located on the roof of the Natural Philosophy building at the University of Melbourne,

Parkville, just north of the central business district. The sampler was placed 14 metres above ground level to conform with Australian Standard AS 2922 – 1987 (Ambient air-guide for the siting of sampling units for environmental monitoring; Standards Australia, Sydney, NSW). Melbourne is situated in the southern hemisphere at a latitude of 38 °S. The climate is temperate, having warm, dry summers and cool wet winters (Ong et al., 1995).

Spore identification

In this study fungi were identified to genus level wherever possible, whereas in our 1992–1994 study we reported total spore numbers and only identified and counted spores of *Cladosporium* and *Alternaria* to genus level (Mitakakis et al., 1997). For this report we re-examined slides collected during 1993, this time identifying as many genera as possible. Absolute spore numbers may vary slightly from our previous report because of the increased number of spore types identified in this re-examination. Calendars were not constructed for spore types with a total annual spore count less than 1000 spores as these levels are too low to demonstrate seasonal distributions.

Spore classification was based on laboratory cultures collected by sampling with a viable volumetric (Andersen) sampler at the same site as the Burkard trap, and reference to Smith (1984, 1986) and CMI descriptions of Pathogenic Fungi and Bacteria. The spore group labelled 'Ascospore 1' was noted in England by Gregory (1973), labelled as 'unknown ascospore'. The unidentified spores of 'Ascospore 2' were approximately $10 \times 5 \mu\text{m}$ in size, brown, kidney shaped, bicellular and have smooth walls.

A number of small, hyaline spores were noted but not counted due to the inability of the Burkard trap to accurately collect particles less than $5 \mu\text{m}$ in aerodynamic diameter (Hirst, 1953). Small-spored fungi include *Aspergillus*, *Penicillium*, *Botrytis*, *Paecilomyces*, *Beauveria*, *Gliocladium*, *Metarrhizium*, *Chaetophoma*, *Dendrophoma*, *Hormiscium*, *Spicaria*, *Verticillium*, *Phoma* and *Nigrospora*, all of which have been isolated from the atmosphere of Melbourne using the viable volumetric Andersen sampler along side the Burkard trap (Mitakakis, 1994). Some of these small spores belong to important allergenic fungi (e.g. *Penicillium*, with spores approximately $1\text{--}2 \mu\text{m}$ in aerodynamic diameter). Despite this limitation the Burkard spore trap remains the most robust sampling technique able to quantitatively detect the

majority of airborne fungal spores over a longitudinal period.

Results

Airspora components

Twenty-nine genera and five spore groups were identified (Table 1). The cumulative annual total of daily spore counts for 1993 was 434,315 removal of units. Spores of the genus *Cladosporium* dominated, providing 41.7% of the total count, three times more frequent than the next most abundant genus. The ten most frequently detected spore groups, forming 84.1% of the total count, were *Cladosporium*, *Leptosphaeria*, *Coprinus*, 'Ascospore 1', *Ganoderma*, *Alternaria*, *Periconia*, *Drechslera*, *Pleospora* and *Epicoccum*. The remaining nineteen spore types were present in low numbers, each less than 0.8% of the annual total.

Seasonal variation

The average daily total fungal spore count for 1993 was 1207 spores m^{-3} of air. The daily count ranged between 7530 spores m^{-3} of air detected on the 2nd February to 96 spores m^{-3} , detected ten days later on the 12th February.

The seasonal distributions of the airborne spore groups detected are illustrated in Figure 1. Most spore types were present throughout the year. *Cladosporium* spores were abundant the entire year with a peak spanning spring, summer and autumn. Spore densities of *Alternaria*, *Drechslera*, *Torula* and *Stemphylium* peaked in spring and summer, whilst spores of *Periconia* were present exclusively in summer. Spore densities of *Leptosphaeria*, *Ganoderma*, *Epicoccum*, *Pithomyces*, *Polythrincium* and 'smuts' peaked toward summer and autumn. Concentrations of spores of *Paraphaeosphaeria* and of 'Ascospore 1' both peaked in winter, whilst spores of *Pleospora* were highest from winter to spring. Numbers of the basidiospores of *Coprinus* and of 'rusts' fluctuated sporadically all year around.

Discussion

The total annual count appears similar to that reported in other countries e.g. Jamaica (Royes, 1987) and Kuwait (Halwagy, 1994). The examination of the components of the total count identified *Cladosporium* as

Table 1. List and percentage contribution of airborne fungal spores in the atmosphere of Melbourne, 1993.

Spore Type	Annual count	Percentage total
<i>Cladosporium</i>	180958	41.7
<i>Leptosphaeria</i>	64735	14.9
<i>Coprinus</i>	63472	14.6
Ascospore 1	63738	5.5
<i>Ganoderma</i>	9303	2.1
<i>Alternaria</i>	6228	1.4
<i>Periconia</i>	5243	1.2
<i>Drechslera</i>	4140	1.0
<i>Pleospora</i>	3723	0.9
<i>Epicoccum</i>	3672	0.8
<i>Paraphaeosphaeria</i>	3171	0.7
<i>Torula</i>	2879	0.7
<i>Pithomyces</i>	1797	0.4
<i>Polythrincium</i>	1293	0.3
<i>Stemphylium</i>	1071	0.2
<i>Xylaria</i>	609	0.14
Ascospore 2	396	0.09
<i>Chaetomium</i>	351	0.08
<i>Sporormiella</i>	303	0.07
<i>Bovista</i>	258	0.06
<i>Curvularia</i>	153	0.04
<i>Leptosphaerulina</i>	144	0.03
<i>Pestalotiopsis</i>	108	0.02
<i>Venturia</i>	69	0.02
<i>Ascobolus</i>	57	0.01
<i>Puccinia</i>	18	<0.01
<i>Fusarium</i>	9	<0.01
<i>Fusariella</i>	9	<0.01
<i>Tetraploa</i>	6	<0.01
<i>Helicoma</i>	6	<0.01
<i>Speggazzinia</i>	3	<0.01
Smuts	42950	9.9
Rusts	13347	3.1
Myxomycetes	96	0.02
Total	434315	100.00

the dominant fungal genus. The dominance of this genus has been observed in many locations, including Austria (Ebner et al., 1992), Denmark (Larsen and Gravesen, 1991), Spain (Infante-Garcia-Pantaleon et al., 1992), Italy (Fillipello Marchisio et al., 1997), Jordan (Shaheen, 1992), India (Singh et al., 1994), Canada (Li and Hsu, 1995), and USA (Sneller and Roby, 1979). In both our investigation and these studies, *Cladosporium* was present year round. Seasonally, summer peak counts of *Cladosporium* were

also observed in other temperate areas including Austria, Denmark and Jordan. Studies in the winter rainfall areas of Italy and California (USA), and in India, found *Cladosporium* spore counts peaked between late autumn and early spring, when temperatures are commensurate with the summer temperatures of cooler regions.

Interestingly, few previous studies have reported *Leptosphaeria* or *Coprinus* spores. The basidiospores of *Coprinus* are unlikely to be identified by viable sampling studies, but even volumetric impaction studies have not previously reported *Coprinus* in such high numbers. *Coprinus* is a common saprophyte favoured by the temperate climates such as in southeast Australia (Shepherd and Totterdell, 1988). *Coprinus* has been listed as a 'frequently collected basidiomycete' in Oklahoma, USA, along with two other basidiomycete genera detected in Melbourne, *Ganoderma* and *Bovista* (Levetin, 1991). Other studies have grouped all basidiospores together. In Cape Town, an examination of spores in the atmosphere over a two week period in winter found basidiospores dominant, comprising more than half of the total spores collected (Hawke and Meadows, 1989). As in our study, in Sardinia (Italy) basidiospores were reported as the second most dominant spore type after *Cladosporium*, peaking in numbers between late autumn and early winter (Palmas and Cosentino, 1990).

Spores of the groups 'rusts', 'smuts', *Fusarium*, *Curvularia*, *Pleospora*, *Epicoccum*, *Torula*, *Helicoma*, and *Puccinia* were previously identified in Melbourne using gravity collection methods by Derrick and Mc Lennan (1963) and later again by Derrick (1965). Using the open plate method in the latter study, they observed a number of genera that were not detected in this study. Many of these spore types were too small to be accurately collected with a Burkard spore trap.

A number of the fungal types detected in Melbourne's atmosphere are widely known allergic triggers. The genera *Cladosporium* and *Alternaria* are the most well recognised of the list (Sward-Nordmo et al., 1984, Yunginger, 1976). Other spore types detected in this study known to contain allergens are *Stemphylium*, *Epicoccum*, *Curvularia* (Shivpuri and Agarwal, 1969), *Drechslera* (Horner et al., 1995), *Coprinus* (de Zubiria et al., 1990), *Ganoderma* (Horner et al., 1995, Tarlo et al., 1979, Vijay et al., 1991b), *Fusarium* (Cosentino et al., 1995), and those of 'rusts' and 'smuts' (Horner et al., 1995, Shivpuri and Agarwal, 1969). The

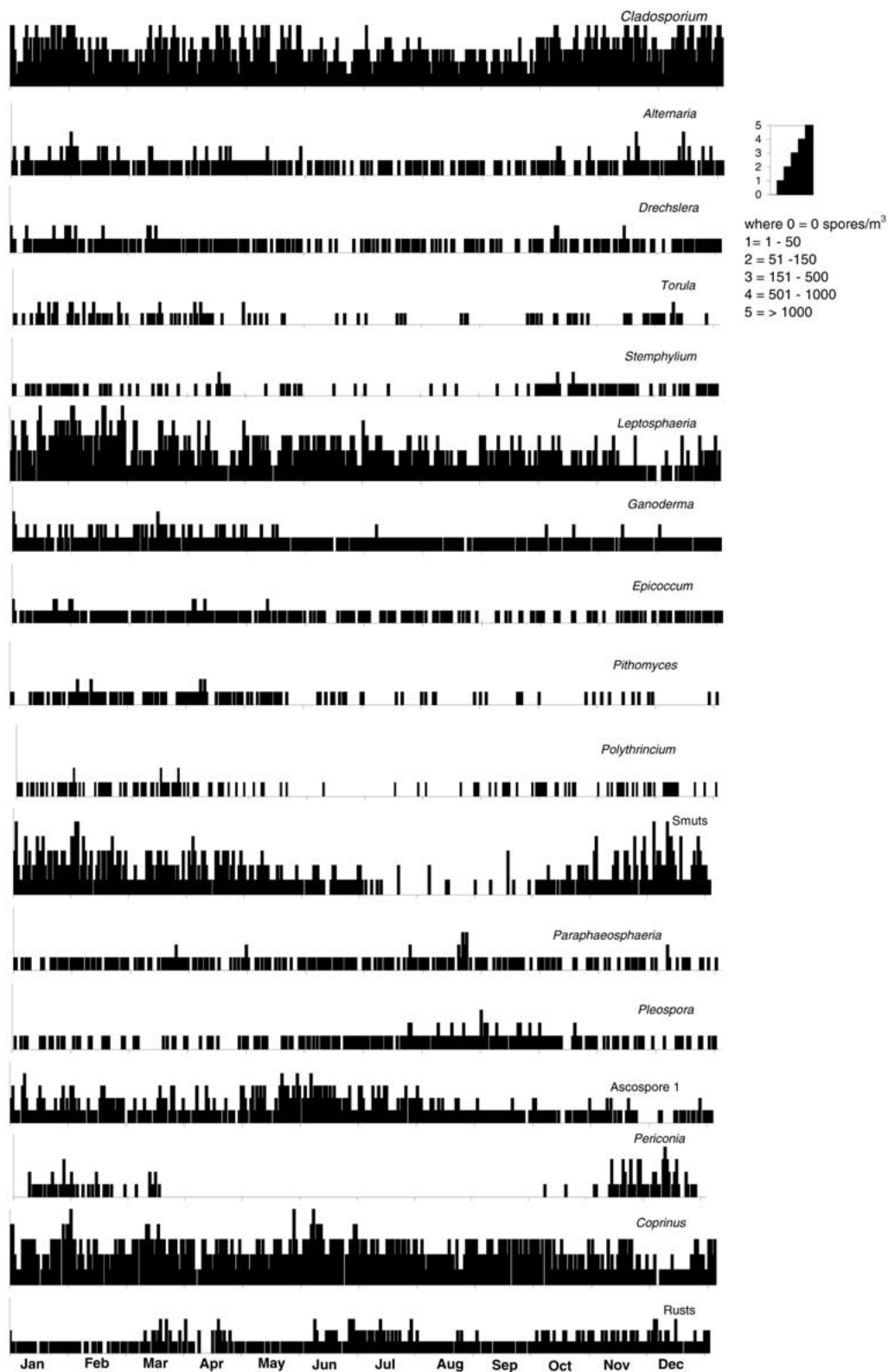


Figure 1. Daily fungal spore calendar for Melbourne, 1993.

remaining fungi detected in Melbourne have not been tested for allergenicity.

A number of these allergenic fungal genera possess cross-reactive allergens which may have implications for the treatment of fungal allergies. Allergens of *Cladosporium* have been demonstrated to cross react with *Alternaria* (Tee et al., 1987; Vijay et al., 1991a). Given that the numbers of *Cladosporium* spores greatly outweigh those of *Alternaria*, this cross reactivity has most importance for those people with sensitisation to *Alternaria*. In Melbourne, the prevalence of sensitisation to *Cladosporium* allergens amongst asthmatics is 32% while 44% are sensitised to allergens of *Alternaria* (Abramson et al., 1994). The higher prevalence of *Alternaria* sensitisation is possibly explained by *Alternaria* allergens cross reacting with a range of genera; those also of *Stemphylium*, *Epicoccum*, *Fusarium*, *Drechslera* and *Curvularia* (Hoffman et al., 1979; Agarwal et al., 1982; Licorish et al., 1985; Verma et al., 1995). *Epicoccum* is further cross-reactive with *Fusarium* (Hoffman et al., 1979). When interpreting the calendar of a single fungus known to trigger a patient's allergic symptoms, the seasonal distributions of all cross reactive fungi must be considered. However, it should be noted that the presence of allergens and of cross reactivities between the remaining reported fungal spore types have not been researched.

Conclusion

A diversity of fungal spores were present in Melbourne's atmosphere in 1993. Total atmospheric spore densities varied seasonally from peak counts in summer to mid winter to troughs from late winter to early spring. A number of allergenic fungal spore calendars are presented which may be used assist the identification and control of fungal allergy symptoms.

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