

Incidence of *Cladosporium*, *Alternaria* and total fungal spores in the atmosphere of Melbourne (Australia) over three years

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Abstract

A Burkard spore trap was used to monitor daily fungal spore counts in the atmosphere of Melbourne (Australia) between October 1991 and December 1994. Annual total spore counts varied widely (range 345 770 in 1994 to 1 106 037 in 1992), of which approximately half were identified as *Cladosporium* sp. and only about 1% *Alternaria* sp. Highest daily total spore counts were recorded late-summer through to mid-winter, probably corresponding to senescence of annual grasses and leaf fall. Spore counts were negatively correlated with rainfall ($P < 0.05$), significantly correlated with average temperature ($P < 0.001$), and showed a highly significant linear relationship with cumulative temperature throughout the year. © 1997 Elsevier Science Ireland Ltd.

Keywords: *Alternaria*; *Cladosporium*; Fungal spore counts; Air monitoring; Aerobiology; Temperature; Rainfall

1. Introduction

Many fungi disperse their spores by releasing them into the turbulent airstreams of the atmosphere, allowing the spores to be distributed some distance away from their source. Airborne spores are important triggers of asthma and hayfever in human health, and Melbourne is a cool temperate city known to have a high incidence of these allergic diseases (Abramson et al., 1994). Sources of airborne allergens include a range of fungi that disperse their spores in air currents, including the morphologically distinct spores of *Cladosporium* and *Alternaria*. These spores may originate from internal and external environments and both contribute to human allergy. This study examines the external environment.

The seasonal quantitative patterns of pollen in the atmosphere of Melbourne have been established dur-

ing the past two decades by Smart and Knox (1979) and Ong et al. (1995). The incidence of fungal spores is less well known. Two earlier studies in Melbourne established the presence of a range of fungal spores throughout the years 1960 and 1965 using the open-plate method (Derrick and McLennan, 1963) and gravity slide technique (Derrick, 1965). Neither study yielded sufficiently detailed information upon which a spore calendar could be constructed. Smart and Knox (1979) compared the quantitative incidence of pollen and spores during a single growing season and observed the occurrence of spores that are characteristically released under dry and wet conditions. The present study documents spore incidence in the atmosphere of Melbourne over 3 years, focusing on the two genera *Cladosporium* and *Alternaria*. An understanding of seasonal patterns in fungal spore number and type may assist in determining the total allergen load of the atmosphere, and thus the risk to sufferers of asthma and hayfever.

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Table 1
Total annual spore counts

Group	1992	1993	1994
Total spores	1 106 037	391 777	345 770
<i>Cladosporium</i>	533 702	180 958	248 862
<i>Alternaria</i>	6995	6228	3951

2. Materials and methods

2.1. Spore counts

Spore counts were collected between January 1992 and December 1994 using a Burkard 7-day volumetric trap with a flow rate of 10 l/min (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK).

Spores were trapped onto adhesive tape (10% Dow Corning A280 series adhesive in xylene on melinex tape) that was cut into segments corresponding to 24 h periods. The tape segments were placed onto separate microscope slides and stained with Calberla's stain for pollen identification (for use in other studies), then viewed by light microscopy for fungal spores. The daily concentrations of aerospores were determined by scanning the middle of the prepared tape, in one lengthwise traverse at a magnification of $500\times$. The spore counts were expressed as the number of spores per m^3 of air according to Kapyla and Penttinen (1981).

2.2. Location of sampler

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

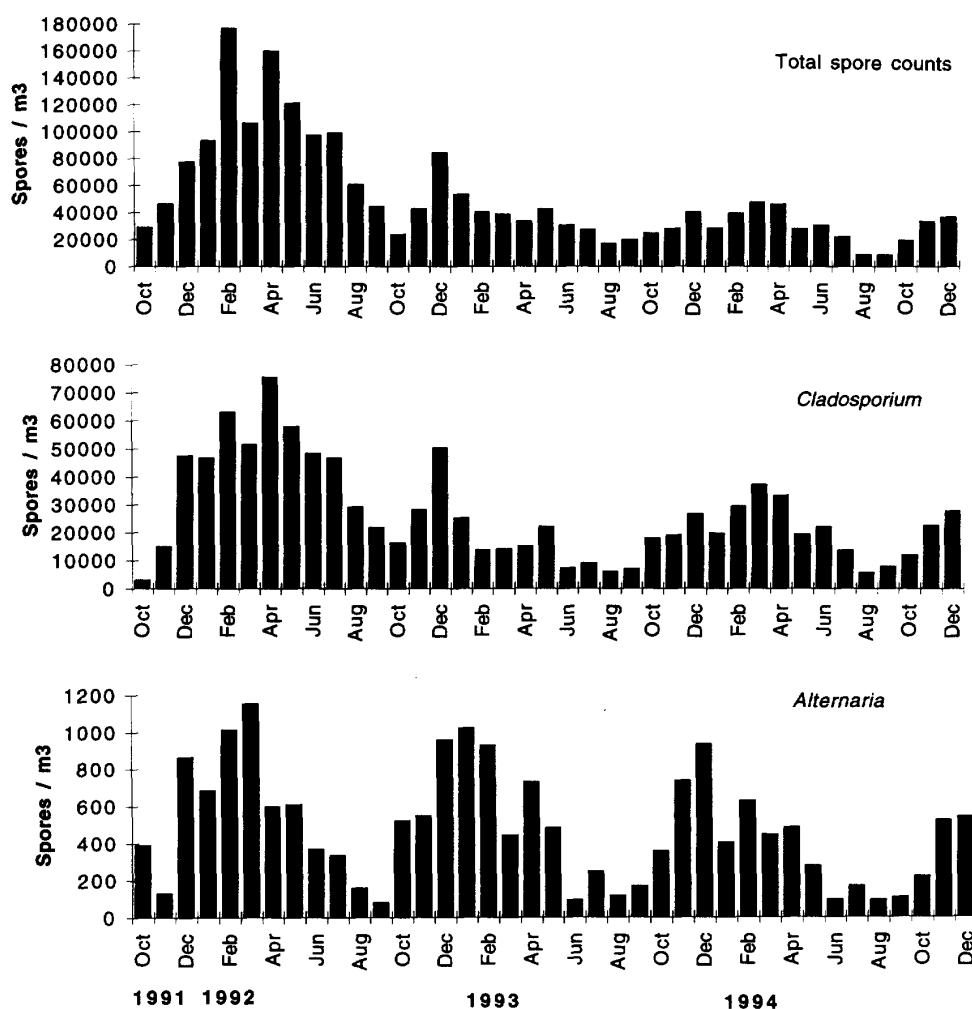


Fig. 1. Monthly spore calendars, based on total daily counts m^{-3} air, for Melbourne from October 1991 to December 1994 showing total spores and data for *Cladosporium* and *Alternaria*.

Table 2

Monthly spore counts for Melbourne, Australia (spores/m³ air) with percentages of the annual total in brackets, 1992–94

Year	Spore group	Jan	Feb	Mar	Apr	May	Jun
1992	Total spores	93 012 (8)	176 541 (16)	105 851 (10)	159 563 (14)	121 122 (11)	96 951 (9)
1993		53 089 (14)	39 901 (10)	38 394 (10)	32 985 (8)	42 447 (11)	30 258 (8)
1994		27 878 (8)	39 138 (11)	47 340 (14)	45 944 (13)	27 555 (8)	30 194 (9)
1992	<i>Cladosporium</i>	46 614 (9)	62 955 (12)	51 318 (10)	75 362 (14)	57 717 (11)	48 231 (9)
1993		24 979 (14)	13 482 (7)	13 920 (8)	14 913 (8)	22 002 (12)	7137 (4)
1994		19 583 (8)	29 118 (12)	37 077 (15)	33 245 (13)	19 164 (8)	21 905 (9)
1992	<i>Alternaria</i>	678 (10)	1011 (14)	1152 (16)	594 (8)	606 (9)	366 (5)
1993		1020 (16)	927 (15)	441 (7)	726 (12)	480 (8)	93 (1)
1994		399 (10)	624 (16)	444 (11)	483 (12)	273 (7)	93 (2)
Year	Spore group	Jul	Aug	Sep	Oct	Nov	Dec
1992	Total spores	99 141 (9)	60 231 (5)	44 145 (4)	23 202 (2)	42 253 (4)	84 025 (8)
1993		26 961 (7)	16 641 (4)	19 221 (5)	24 222 (6)	27 516 (7)	40 142 (10)
1994		21 633 (6)	8487 (2)	8471 (2)	18 897 (5)	33 471 (10)	36 762 (11)
1992	<i>Cladosporium</i>	46 689 (9)	29 034 (5)	21 618 (4)	16 056 (3)	27 983 (5)	50 125 (9)
1993		8853 (5)	5733 (3)	6723 (4)	17 805 (10)	18 924 (10)	26 487 (15)
1994		13 551 (5)	5523 (2)	7725 (3)	11 811 (5)	22 374 (9)	27 786 (11)
1992	<i>Alternaria</i>	333 (5)	156 (2)	78 (1)	519 (7)	546 (8)	956 (14)
1993		246 (4)	114 (2)	165 (3)	354 (6)	732 (12)	930 (15)
1994		165 (4)	90 (2)	105 (3)	216 (5)	522 (13)	537 (14)

Parkville, just north of the central business district. The sampler was placed 14 m above ground level to conform with Australian Standard AS 2922-1987 (Ambient air-guide for the siting of sampling units for environmental monitoring; Standards Association of Australia, Sydney, NSW).

2.3. Spore classification

Spores were identified by reference to cultures collected using an Andersen sampler at the same site as the Burkard trap. Additional information on fungal identification came from Smith (1984, 1986) and CMI descriptions of Pathogenic Fungi and Bacteria. Fungi were identified to genus level.

2.4. Statistical analysis

To investigate the correlations between the daily spore counts and weather parameters, the data were compiled in Microsoft Excel v.5.0 spreadsheets (Microsoft Corp., USA) and analyzed using this software or using Minitab v.10 Xtra (Minitab Inc., State College PA, USA). For Rank correlation coefficients, the parameters examined included daily rainfall and mean daily temperature using data obtained from the National Climate Centre, Bureau of Meteorology, Melbourne. The level of significance was assessed between $P < 0.05$ to $P < 0.001$. Regression analyses and linear regression plots were also carried out using these data.

3. Results

3.1. Comparison between annual spore counts

There were considerable differences in the total annual spore counts between the 3 years monitored. Thus counts for 1992 were nearly 3 times those found in succeeding years, giving a ratio of 3.2:1.1:1 (1992:1993:1994; Table 1).

Annual totals for *Cladosporium* gave a ratio of 2.1:0.7:1 for the same years (Table 1). *Cladosporium* was by far the most common spore detected, amounting to 48.3% of the total spore catch for 1992, 46.2% for 1993 and 71.8% for 1994. The annual total of *Alternaria* spores gave a ratio of 1.8:1.6:1 over the 3 years, varying from 0.6% of total spore catch in 1992, 1.5% in 1993 to 1.1% in 1994 (Table 1).

3.2. Seasonal patterns

Despite the differences in total annual spore numbers, seasonal patterns were similar. Peak spore counts occurred from December to June while spore counts in August and September were consistently low (Fig. 1, Table 2). Overall, the summer and autumn months contributed 66% of the spore catch (averaged over 3 years), while winter contributed 21% and spring only 13% (Fig. 2).

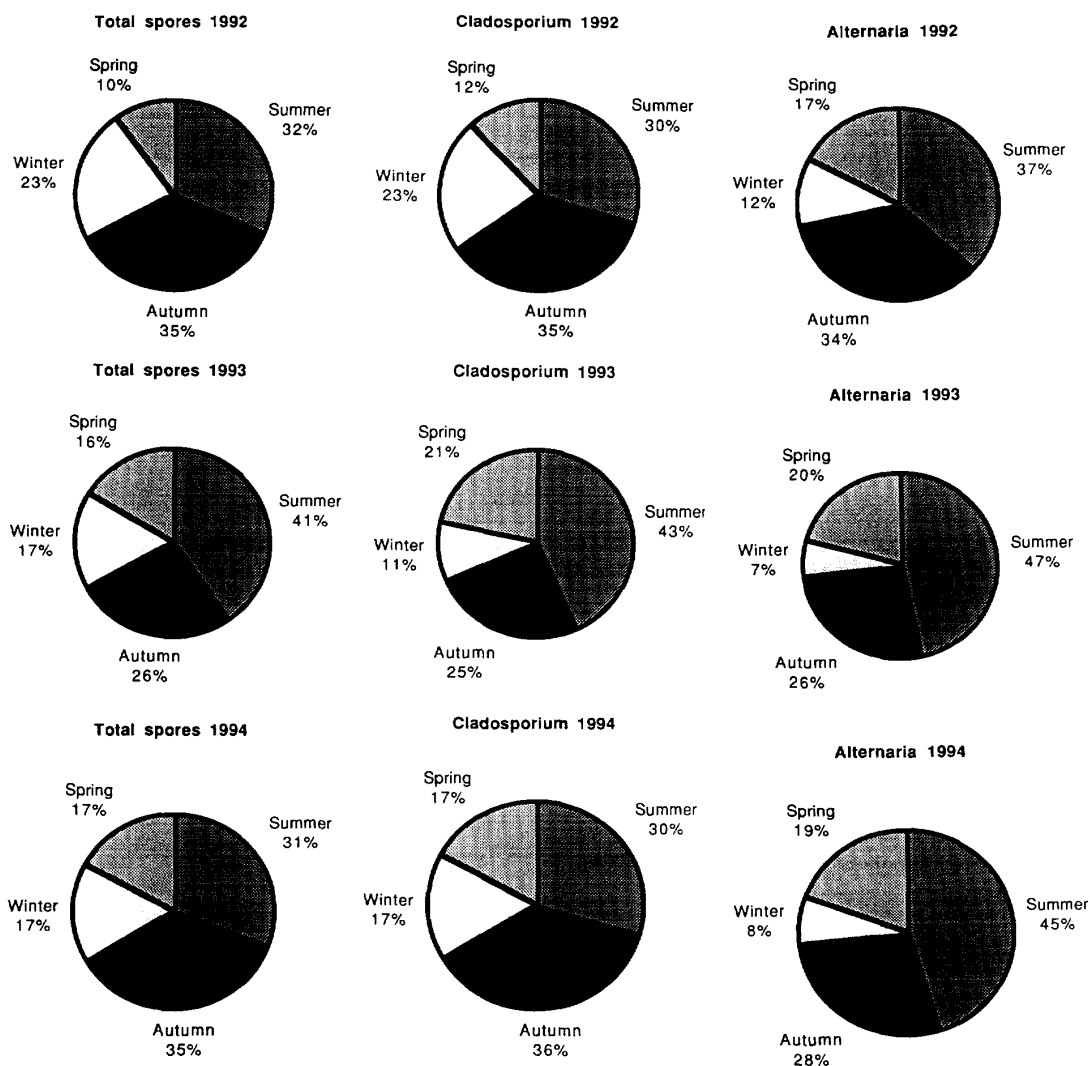


Fig. 2. Charts showing seasonal distribution of spores in Melbourne for the years 1992, 1993 and 1994. Data includes total spores, *Cladosporium* and *Alternaria* based on total daily counts m^{-3} air. Summer includes 1 December to 28 February; Autumn, 1 March to 30 May; Winter, 1 June to 31 August; Spring, 1 September to 30 November.

As *Cladosporium* was the largest single genus of spores in the annual catch, its distribution usually paralleled the distribution of total spores (Fig. 1). In the 1991/1992 season, highest counts were from December to July. In 1992/1993, counts peaked in December only, while in the succeeding year, counts were low but peaked in March 1994.

Alternaria spores showed a regular pattern of seasonal variation (Fig. 1). Peaks generally appeared bimodal, with a peak in early summer (December) followed by a second seasonal peak in autumn (March and/or April). In 1993/1994, November and December

were equally high, with the second peak in February rather than later.

3.3. Daily fluctuations

Spores were detected in the atmosphere of Melbourne for every day of the 3 years (Fig. 3). The total daily counts fluctuated widely, ranging from a low of 21 to a high of 10 263 spores/ m^3 in 1992, 60–6345 spores/ m^3 in 1993, and 66–4023 spores/ m^3 in 1994. Mean total daily counts varied from 3030 spores/ m^3 in 1992, 1071 in 1993 to 947 in 1994.

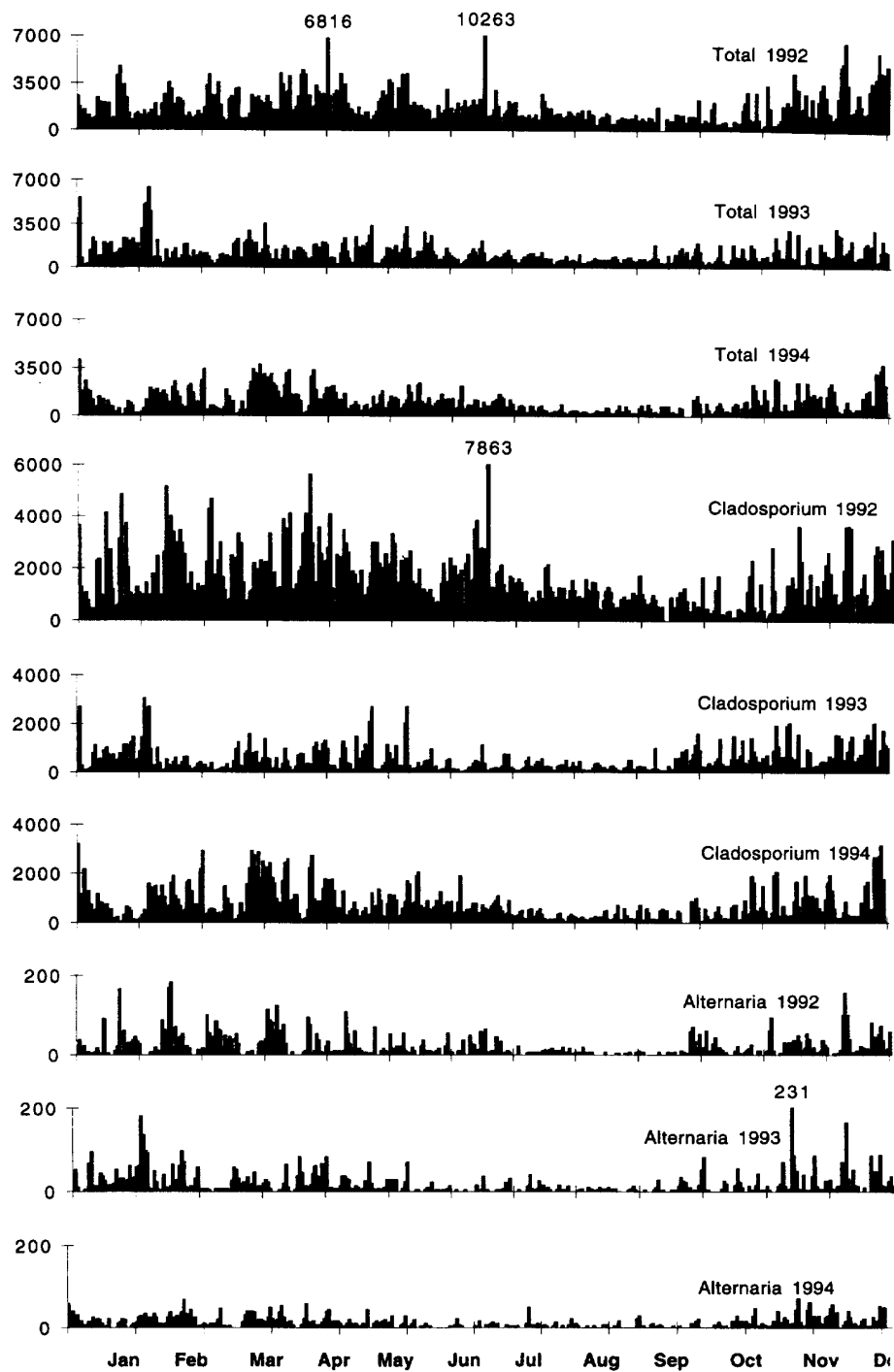


Fig. 3. Spore calendars for Melbourne for the years 1992, 1993 and 1994, based on daily counts m⁻³ air for total spores, *Cladosporium* and *Alternaria*, showing the dramatic variations from year to year in the spore catch and seasonal effects.

3.4. Statistical analyses of spore counts and weather parameters

A comparison using a nonparametric method, Spearman Rank correlation coefficient, of daily spore counts shows that they are negatively correlated with rainfall ($P < 0.05$ to $P < 0.001$ according to the year and spore type) and highly positively correlated with average temperature ($P < 0.001$) (Table 3). During 12 of the 19 days between 27 February to 18 March, 1994, very low spore counts coincided with rainfall. From November to January, 1993/94, *Alternaria* counts also appeared to be dictated by the weather patterns. *Alternaria* and *Cladosporium* have 'dry spores' meaning that dry weather is favoured for sporulation. For example, *Alternaria* spore counts were highest in 1993 on the 16 November, when temperatures were mild (20.8°C), with a relative humidity of 60% and no rainfall. By comparison, on November 23 there was a zero *Alternaria* spore count, a temperature maximum of 18°C, with a relative humidity of 46% and rainfall that day and the preceding day.

Cumulative spore counts were regressed against cumulative temperatures (or degree days) for 1992–93 and 1993–94, commencing at the beginning of the thermal season in winter (July). There is a highly significant linear relationship between temperature and total spores, *Cladosporium* and *Alternaria* for both years. These data suggest that the increase in spore number occurs linearly with temperature throughout the year. Accordingly, the data were further analyzed for the 3 calendar years 1992, 1993 and 1994 (Fig. 4) again revealing a highly significant linear regression between daily spore numbers and average temperature. There are no consistent troughs or peaks in the 3 years examined, although there are dramatic differences in the very high counts for total spores and *Cladosporium* in 1992 compared with the other 2 years.

Table 3
Spearman Rank correlation coefficients for the relationships between the daily incidence of total fungal spores, *Cladosporium* and *Alternaria*, and the daily average temperature (°C) and rainfall (mm) for Melbourne, Australia

Spore type	Year	Rainfall	Temperature
<i>Cladosporium</i>	1992	–0.156**	0.233***
	1993	–0.253***	0.193***
	1994	–0.209***	0.325***
<i>Alternaria</i>	1992	–0.112*	0.398***
	1993	–0.279***	0.329***
	1994	–0.271***	0.434***
Total	1992	–0.138**	0.274***
	1993	–0.206***	0.190***
	1994	–0.184***	0.337***

*Indicates $P < 0.05$ ($n = 364$, critical value = 0.086).

**Indicates $P < 0.005$ ($n = 364$, critical value = 0.134).

***Indicates $P < 0.001$ ($n = 364$, critical value = 0.161).

4. Discussion

Cladosporium and *Alternaria* are considered two of the most important fungal sources of allergens and have been the focus of previous research. A case study in Melbourne examining asthmatic and control patients found 44% to have positive skin reactions to *Alternaria* and 32% to *Cladosporium* (Abramson et al., 1994).

In this, and other studies, *Cladosporium* was the most common spore identified, while *Alternaria* spores were less common. This trend is independent of environment, and has been reported in temperate climates (Nussbaum, 1991; Beaumont et al., 1985; Goretti et al., 1991), and the wet (Satheesh and Rao, 1994), monsoonal (Singh et al., 1994) and the dry tropics (Shaheen, 1992). These studies used a variety of air sampling techniques, including the open plate method, Andersen sampler, aeroscope and Burkard 7-day volumetric trap.

The results of this study confirm earlier studies in Melbourne by Derrick and McLennan (1963) and Derrick (1965) which found both *Cladosporium* and *Alternaria* to be present every month of the year. Their results also agreed with peak spore periods in December and January for both genera studied. The air sampling techniques applied in these studies were, however, non-volumetric and used inadequate sampling.

We find that rainfall and cooler temperatures coincided with sudden decreases in *Cladosporium* spore numbers. Rain collects spores and other particulate matter in its path, effectively cleansing the air (Nishihara et al., 1989). The daily spore count fluctuated considerably from one day to the next. A number of studies attribute these fluctuations to weather parameters (Hawke and Meadows, 1989; Madelin and Johnson, 1992; Hasnain, 1993; Hjelmroos, 1993). In this study, we find that the spore counts are negatively correlated with rainfall, and highly significantly correlated with average temperature. A novel finding is the linear relationship between cumulative spore counts and cumulative temperature. As the linear regression lines intercept at the origin, the slope of the lines gives a striking indication of the cumulative spore count for the particular year, and would enable prediction of the annual count. These data are very different from comparable studies of grass pollen counts, where cumulative counts are linear during the flowering season and rapidly tail off at the end of the pollen season (Antepara et al., 1995; R.B. Knox, unpublished data).

The seasonal pattern of *Cladosporium* and *Alternaria* reflects the amount of substrata present, assumed to be associated with senescent leaves of annual grasses and leaf fall as well as favourable weather conditions. *Cladosporium* is soil borne and airborne, and is saprophytic on dead wood, leaf debris and grasses. *Alternaria*, despite being largely a soil borne fungus, prefers rotting organic matter such as wood, tobacco,

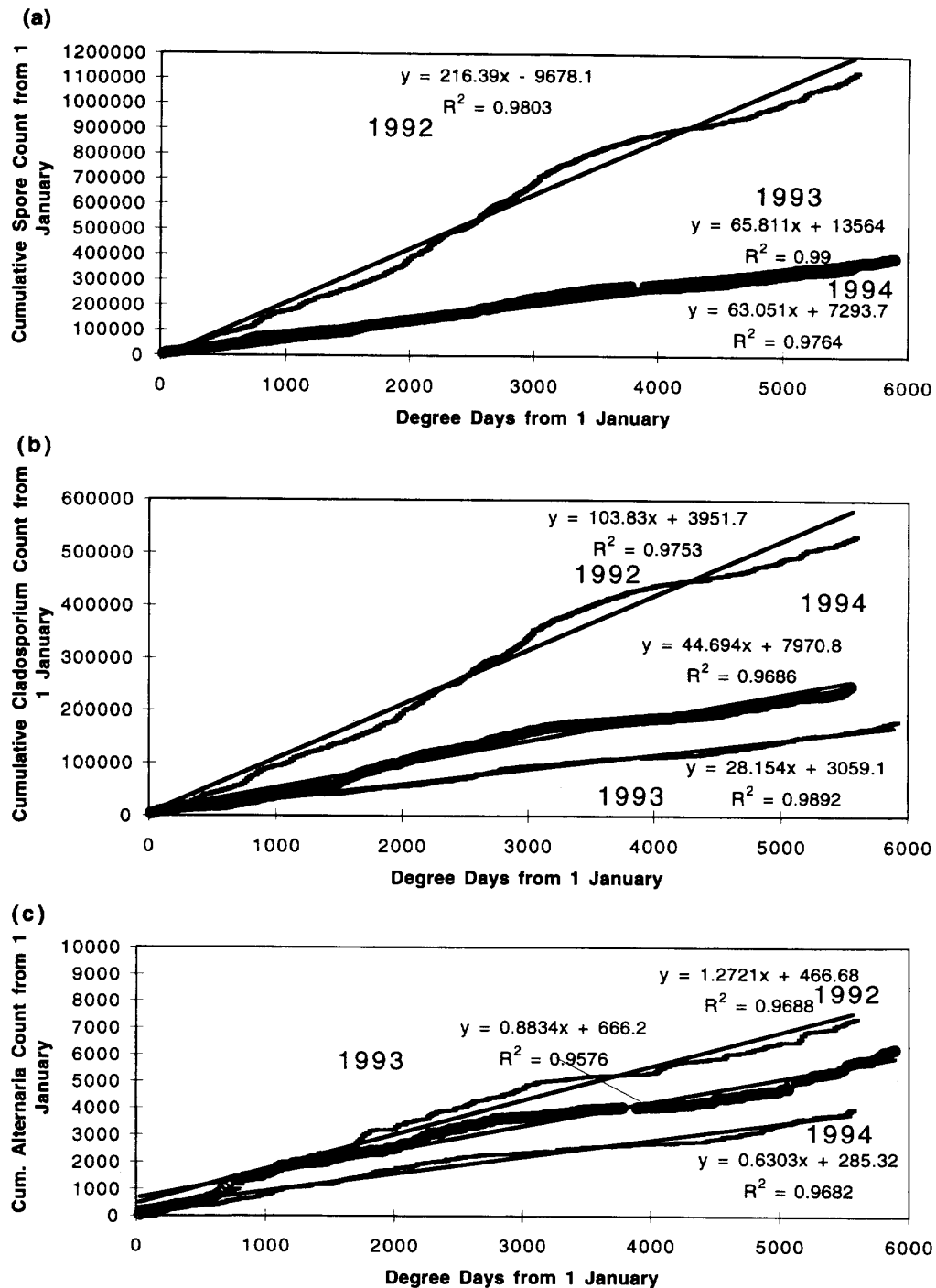


Fig. 4. Scatter plots of the cumulative spore counts (from 1 January to 31 December) and temperature (degree days from 1 January to 31 December) showing the significant linear relationship between these variables. (a) Shows total spores for 1992, 1993 and 1994; (b) shows *Cladosporium* spores for 1992, 1993 and 1994; (c) shows *Alternaria* spores for 1992, 1993 and 1994.

potato, leaf debris, and, like *Cladosporium* is also found on grasses. Melbourne has many deciduous trees and annuals planted in parks and gardens, and has extensive grasslands near its outer suburbs and cereal crops to the north, north-west and south-east. These plants have rapid growth and turnover in summer followed by senescence in autumn and winter and produce large amounts of dead organic matter that *Cladosporium* and *Alternaria* colonise. The seasonal pattern of these two genera closely follows the life cycle of the local vegetation.

5. Conclusions

Our results show that in Melbourne, annual total spore counts varied from 345 770 in 1994 to 1 106 037 in 1992. Between 46 and 72% of spores counted proved to be *Cladosporium* and between 0.6 and 1.5% *Alternaria*. Highest total spore counts were recorded late-summer through to mid-winter, probably corresponding to the death of annual grasses and leaf fall. Spore counts were negatively correlated with rainfall ($P < 0.05$), significantly correlated with average temperature ($P < 0.001$), and showed a highly significant linear relationship with cumulative temperature throughout the year. Further research will focus on producing a more detailed calendar showing the incidence and types of fungal spore genera present in the Melbourne atmosphere, and to the level of mould spores in indoor environments.

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