



The long-term trends and seasonal variation of the aeroallergen *Alternaria* in Derby, UK

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Abstract

Very little is known in the UK about long term trends of the *Alternaria* spore although it is known to trigger asthma. It has recently become apparent that *Alternaria* spore levels are increasing in Derby and a detailed study of *Alternaria* was undertaken to investigate the increase in numbers, seasonal variation and diurnal periodicity. The seasonal (June–October) *Alternaria* spore concentrations show a distinct upward trend and there is evidence of an earlier seasonal start and an increase in the seasonal duration. There has been a dramatic rise in the number of days with an *Alternaria* spore count above 50 spores per cubic metre, with the peak daily count usually occurring in August but occasionally in late July or early September. August generally has the highest monthly total and for 1991–1998 there was a positive correlation with monthly rainfall and average temperature. Day to day spore levels show a positive correlation between *Alternaria* spore concentrations and maximum temperature but a slight negative correlation with daily rainfall. The peak time for spore capture is 14.00–22.00, and more than half the daily *Alternaria* catch is caught between 18.00 and 24.00 hours. The upward trend in *Alternaria* spore concentrations may be responsible for increasing levels of respiratory disease, especially during harvest time.

Introduction

Certain fungal spores are known to cause an allergic response and spores of the genus *Alternaria*, a member of the Deuteromycetes, have been known to trigger asthma for over 50 years (Hopkins et al., 1930). The risk of asthma-related deaths is more than doubled when there is a high incidence of mould spores (Targonski et al., 1995) and *Alternaria* is a known risk factor in childhood and young adult asthma (O'Hollaren et al., 1991; Tariq et al., 1996).

Alternaria is found everywhere, it is an extremely common saprophyte found in the soil, on plants, and in the air. The spore is present in large numbers throughout the late summer, especially at harvest time (Hyde and Williams, 1946). Aerobiological monitoring of both pollen and spores has been taking place in Derby since 1970 and it has recently become apparent

that *Alternaria* spore levels are increasing. There have been no long term studies of airborne concentrations of *Alternaria* spores in the UK and so a detailed study was undertaken to investigate the increase in numbers, seasonal variation and diurnal periodicity.

In this study we have only considered *Alternaria* in the outdoor air, as previous work by us has shown that in houses with no indoor source of *Alternaria*, only very small numbers of this spore penetrate from the outside, especially if there is double glazing (Stern et al., 1999). In rural Australia the lowest concentrations of *Alternaria* spores were also found indoors (Mitakakis et al., 1998).

Alternaria spores vary in size but Licorish et al. (1985) said it could be safely assumed that even the larger *Alternaria* spores could penetrate the lungs causing asthma. If more *Alternaria* spores are being released into the air, even though only a

small percentage of the population is involved, then a considerable number of patients may have severe asthma attacks during the *Alternaria* season.

Materials and methods

Derby is the third largest city in the East Midlands, with a population of about 230,000. It is located on the River Derwent, to the south and east of the Derbyshire uplands, and on the northern flanks of the Trent valley. Derby is an industrial city more than 100 km from any coast and is surrounded on three sides by large areas of arable farming.

A volumetric spore trap (Burkard Manufacturing Co. Ltd, Rickmansworth, UK.) is sited on the roof of Derby University Mickleover site, 10 m above ground level, in a suburb 100 m above sea level and approximately 4 km SW of the city centre. A meteorological station 0.5 km from the present trap site provides weather data. From 1970–1989 a Morrow Brown trap (Morrow Brown and Jackson, 1978) was sited on a second storey roof 10m above ground level in central Derby, building developments necessitated the site change to Mickleover in 1990 which has improved accessibility and maintained optimum sampling height. In 1991 the Morrow Brown trap, previously found to be comparable with a Hirst trap for *Alternaria* spores (Morrow Brown and Jackson, 1978), was replaced by a Burkard volumetric spore trap. Spore monitoring has taken place throughout the whole year since then. Daily slide or seven day drum traps were used, and mounting and staining techniques followed the methods described by Morrow Brown and Jackson (1978). All sizes of *Alternaria* spores were counted as the spore size varies greatly between species e.g. *Alternaria alternata* is much smaller than *Alternaria brassicae*. Average daily concentrations (spores m⁻³) were calculated by counting one microscope field along the length of the slide and applying the appropriate conversion factor. From 1986 to 1988 a second trap, modified to sample for one six minute period every hour was used and hourly concentrations were calculated from the counts of three equidistant longitudinal strips of each hourly sample. These June–October 1986–1988 hourly concentrations were averaged and used to establish diurnal periodicity. Eight hour frequencies were also determined.

The *Alternaria* season in Derby is from June to October although a few *Alternaria* spores are found throughout the year, so June–October seasonal

totals were calculated for each year from 1970–1998 and plotted to assess any long term trend. Monthly *Alternaria* totals and spore level frequencies were also studied for all years. From the seasonal totals, the start, end and duration of season were calculated. Five year running means were also calculated for start dates. The method used was that defined as the date by which 2.5% of the total June–October spore census for the year was trapped, this method has been used with Derby April–July *Quercus* pollen data (Corden and Millington, 1999) and the end of season was defined as that day when 97.5% of the total June–October *Alternaria* spores was reached. The duration of the season is the difference between the start and the end of the season. Trends for seasonal total, start, end, and duration of season were calculated and plotted. The Sum 75 method, previously used for pollen (Driessen et al., 1990) was also used to identify the start of the *Alternaria* spore season, this was the day, from the 1st of June, when the cumulative average daily spore count reached a total of 75 spores per cubic metre of air.

Seasonal, monthly and daily mean concentrations of *Alternaria* spores were compared with meteorological data and selected statistical analyses were carried out.

Results

The seasonal (June–October) *Alternaria* spore concentrations from 1970–1998 (Figure 1) showed a distinct upward trend, which started gradually from 1970–1991 and then increased markedly from 1992 onwards. 1997 was by far the highest season followed by 1992 and 1996. Five year running mean start dates for *Alternaria* showed there was a trend for an earlier seasonal start using both Sum 75 running means (Figure 2) and the 2.5% method, and seasonal duration varied from 67 days in 1984 to 127 days in 1990 the trend being for a longer season (Table 1).

Within the *Alternaria* seasons the number of days when the *Alternaria* count was above 50 spores per cubic metre increased dramatically since 1989, with a maximum daily count of 4292 per cubic metre on 7 September 1996. The peak day per year usually occurred in August but was occasionally found at the end of July or early September (Table 1), August generally had the highest monthly totals (Figure 3) whether the season as a whole was low e.g. 1987 or very high as in 1997. However in 1992 and occasion-

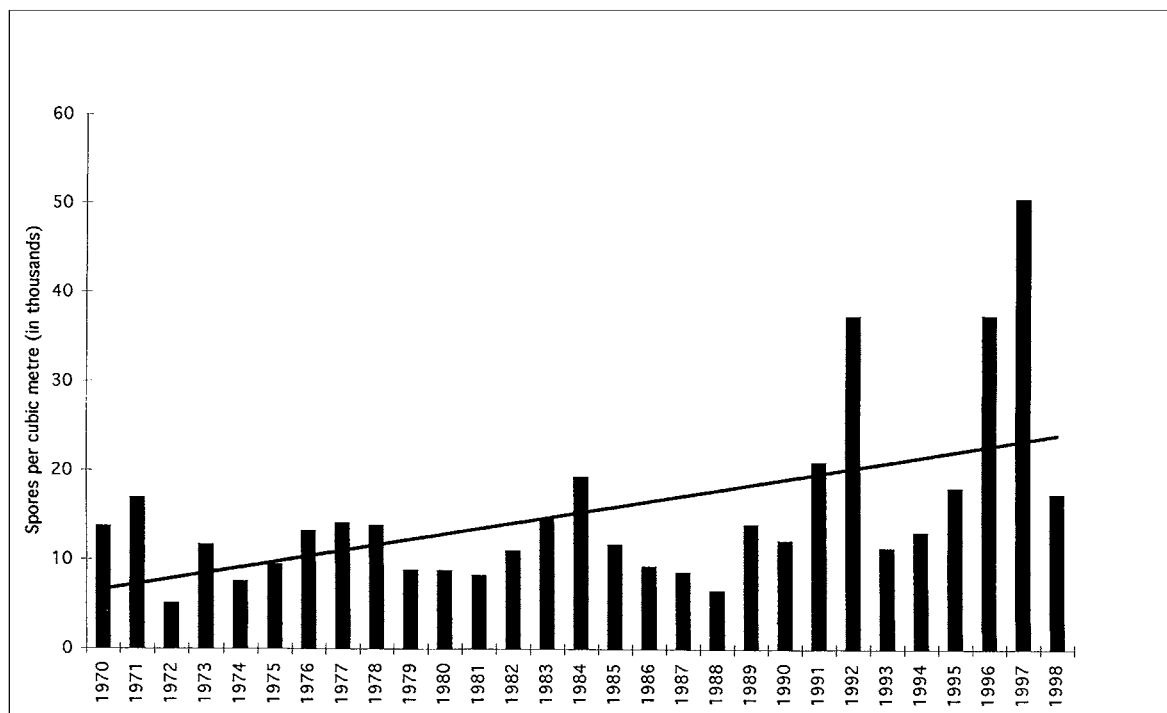


Figure 1. The 1970–1998 seasonal (June–October) *Alternaria* spore concentrations.

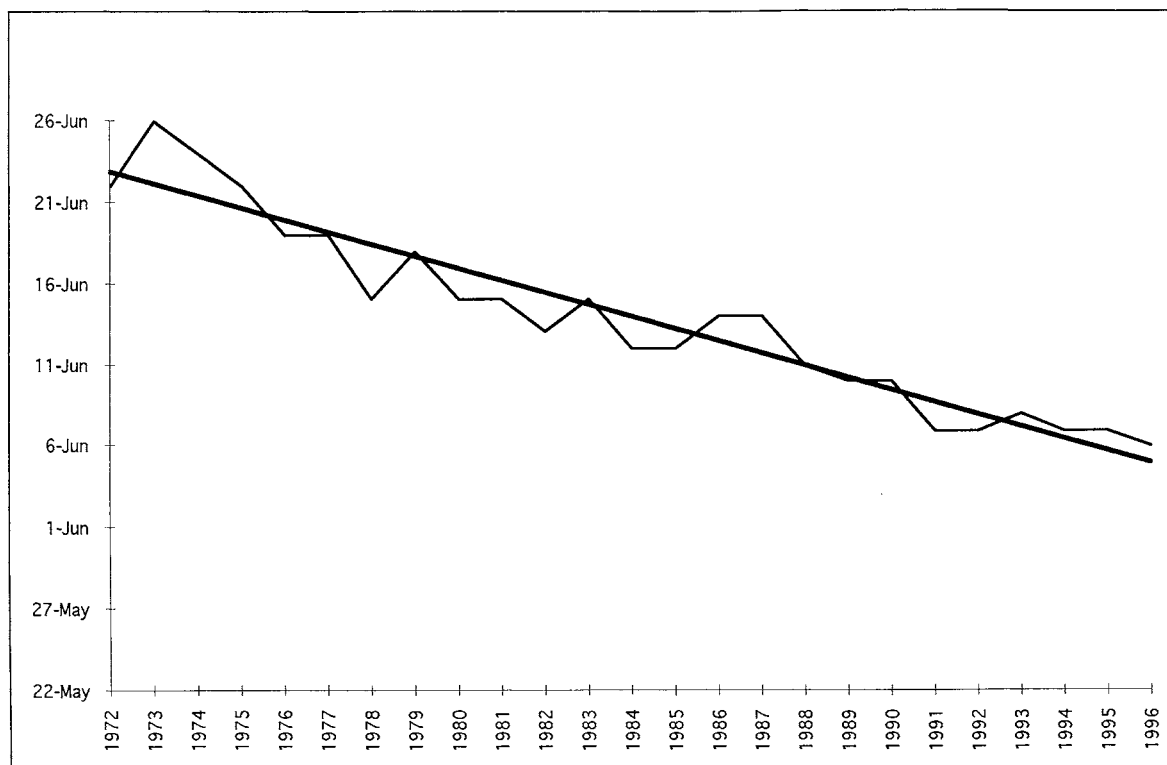


Figure 2. The five year running mean *Alternaria* start date (sum 75 method).

Table 1. Descriptive statistics of *Alternaria* spore seasons 1970–1998.

Year	Seasonal data						Number of Days							Spores
	Start date	Start date	End date	Duration	Peak	Peak	50–99	100–199	200–499	500–999	1000–1999	2000–4500	50 spores	Seasonal
	SUM 75	2.5%	2.5%		value	date	spores	spores	spores	spores	spores	spores	and over	totals
1970	13-Jun	06-Jul	30-Sep	86	1415	28-Aug	20	5	15	7	1	0	48	13705
1971	23-Jun	06-Jul	02-Oct	88	1800	18-Aug	25	11	8	6	4	0	54	16930
1972	30-Jun	10-Jul	14-Oct	96	385	12-Aug	18	8	5	0	0	0	31	5135
1973	23-Jun	30-Jun	01-Oct	93	1060	15-Aug	21	15	9	5	1	0	51	11650
1974	20-Jun	08-Jul	18-Oct	102	930	07-Aug	14	11	10	1	0	0	36	7585
1975	03-Jul	14-Jul	23-Sep	71	890	28-Aug	12	9	12	3	0	0	36	9395
1976	12-Jun	25-Jun	23-Sep	90	800	23-Aug	23	30	15	3	0	0	71	13160
1977	22-Jun	05-Jul	08-Oct	95	2070	16-Aug	15	13	16	2	1	1	48	14105
1978	08-Jun	11-Jul	14-Oct	91	1700	19-Aug	16	19	17	3	1	0	56	13810
1979	19-Jun	11-Jul	26-Sep	95	1415	30-Aug	12	12	6	2	1	0	33	8815
1980	12-Jun	04-Jul	23-Sep	84	575	28-Jul	19	13	11	3	0	0	46	8760
1981	29-Jun	10-Jul	17-Sep	75	725	28-Aug	11	12	13	1	0	0	37	8238
1982	07-Jun	20-Jun	28-Sep	89	900	03-Aug	28	16	13	2	0	0	59	11014
1983	07-Jun	11-Jul	14-Sep	79	1445	19-Aug	17	11	17	4	2	0	51	14525
1984	10-Jun	09-Jul	02-Oct	67	2280	20-Aug	11	15	12	7	3	1	49	19355
1985	20-Jun	04-Jul	04-Oct	90	990	25-Jul	19	21	11	3	0	0	54	11688
1986	17-Jun	28-Jun	03-Oct	98	1405	12-Aug	13	14	5	2	1	0	35	9227
1987	06-Jul	26-Jun	03-Oct	99	1510	20-Aug	21	15	8	0	1	0	45	8595
1988	18-Jun	29-Jun	26-Oct	119	320	07-Sep	28	10	7	0	0	0	45	6615
1989	11-Jun	17-Jun	04-Oct	109	735	06-Aug	18	30	17	3	0	0	68	13970
1990	04-Jun	17-Jun	22-Oct	127	700	24-Aug	33	27	13	1	0	0	74	12124
1991	09-Jun	04-Jul	12-Oct	100	2085	29-Jul	24	27	26	7	0	1	85	21030
1992	06-Jun	21-Jun	16-Sep	87	2465	19-Aug	24	19	22	11	6	3	85	37351
1993	06-Jun	13-Jun	03-Oct	112	552	18-Aug	27	27	12	1	0	0	67	11291
1994	10-Jun	28-Jun	30-Sep	94	735	22-Aug	19	25	14	3	0	0	61	13048
1995	08-Jun	30-Jun	12-Oct	104	1400	01-Aug	22	21	20	7	1	0	71	18082
1996	06-Jun	11-Jul	10-Oct	91	4292	07-Sep	23	17	24	10	7	3	84	37427
1997	03-Jun	01-Jul	20-Sep	81	3145	16-Aug	17	23	17	11	15	3	86	50608
1998	05-Jun	20-Jun	21-Oct	123	1176	31-Aug	29	26	16	5	2	0	78	17388
Range				60 Days										

ally in other years, *Alternaria* spore levels for July were nearly as high as August, but in 1996 levels were higher in September than August, the only occasion since 1970.

On considering the exceptionally high year of 1997 (Figure 4) *Alternaria* spore peaks can be seen in relation to daily maximum temperature and daily rainfall, the highest *Alternaria* peaks occurring on dry days. The June–October 1997 scatter plots (153 data points) of daily average spore concentration (Figure 5) positively correlated with daily maximum temperature (correlation coefficient 0.58, $R^2 = 0.33$) and were slightly negatively correlated with daily rainfall (correlation coefficient -0.18 , $R^2 = 0.03$).

When considering *Alternaria* totals for August (1991–1998), we found that in 1997, both the mean daily temperature for August and the monthly rainfall were high, but in 1992 (the second highest *Alternaria* count) rainfall was plentiful although the mean daily

temperature was low. (Table 2). Multiple regression analysis of August *Alternaria*, rainfall and mean daily temperature gave multiple $R = 0.95$, $R^2 = 0.92$.

The hourly 1986–1998 *Alternaria* spore diurnal periodicity (Figure 6) shows the peak time for spore capture was from 14.00–22.00 hours and more than half the daily *Alternaria* spore catch were caught between 18.00 and 24.00 hours.

Discussion

It has been known for many years that spores of the genus *Alternaria* cause asthma (Hopkins et al., 1930; Frankland and Davies, 1965; Licorish et al., 1985; O'Hollaren et al., 1991; Tariq et al., 1996), with Buisseret (1976) finding *Alternaria* the commonest fungal spore to cause seasonal allergy in the late summer and early autumn and so the increasing

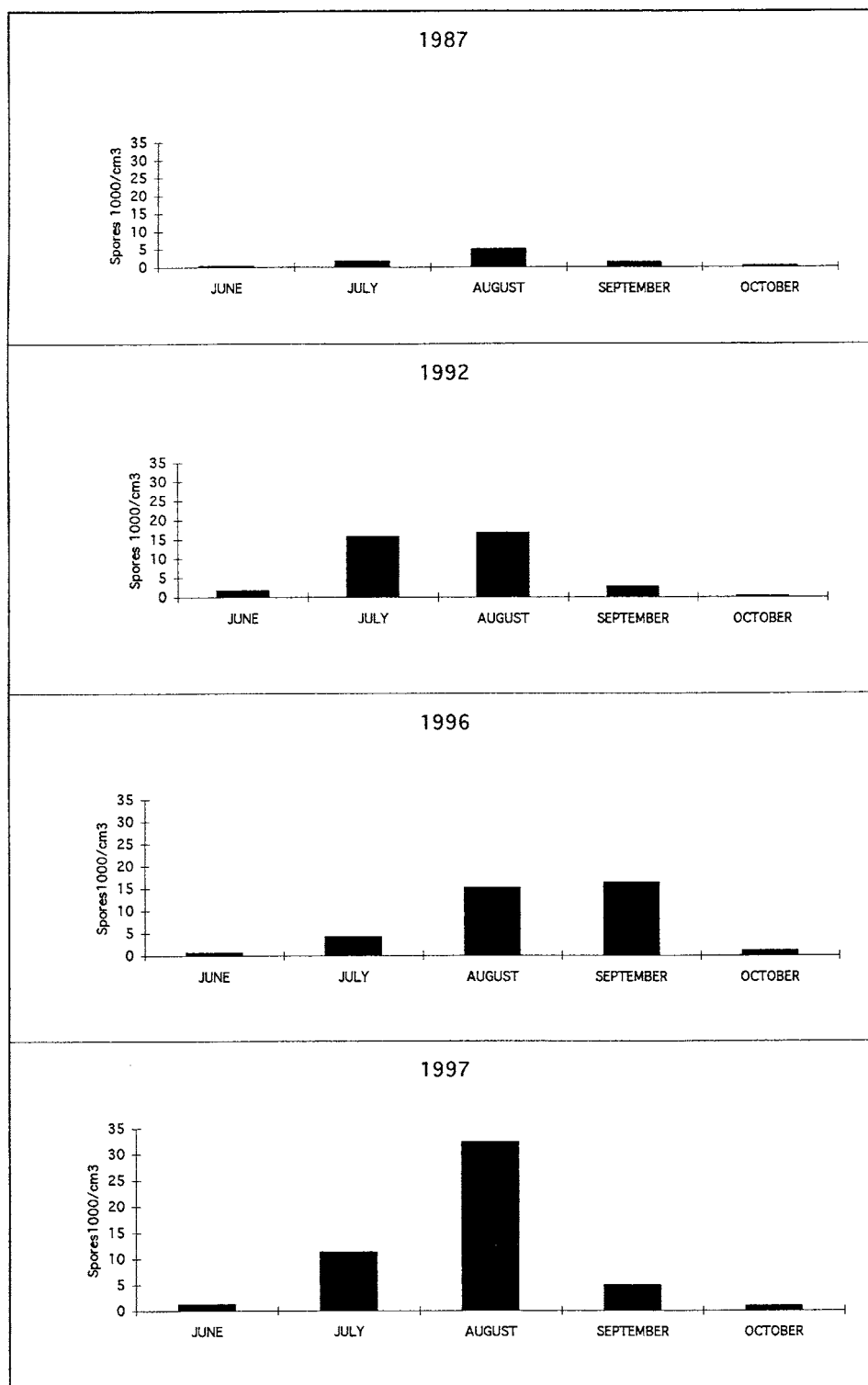


Figure 3. Differing patterns of monthly *Alternaria* spore concentration found in recent years.

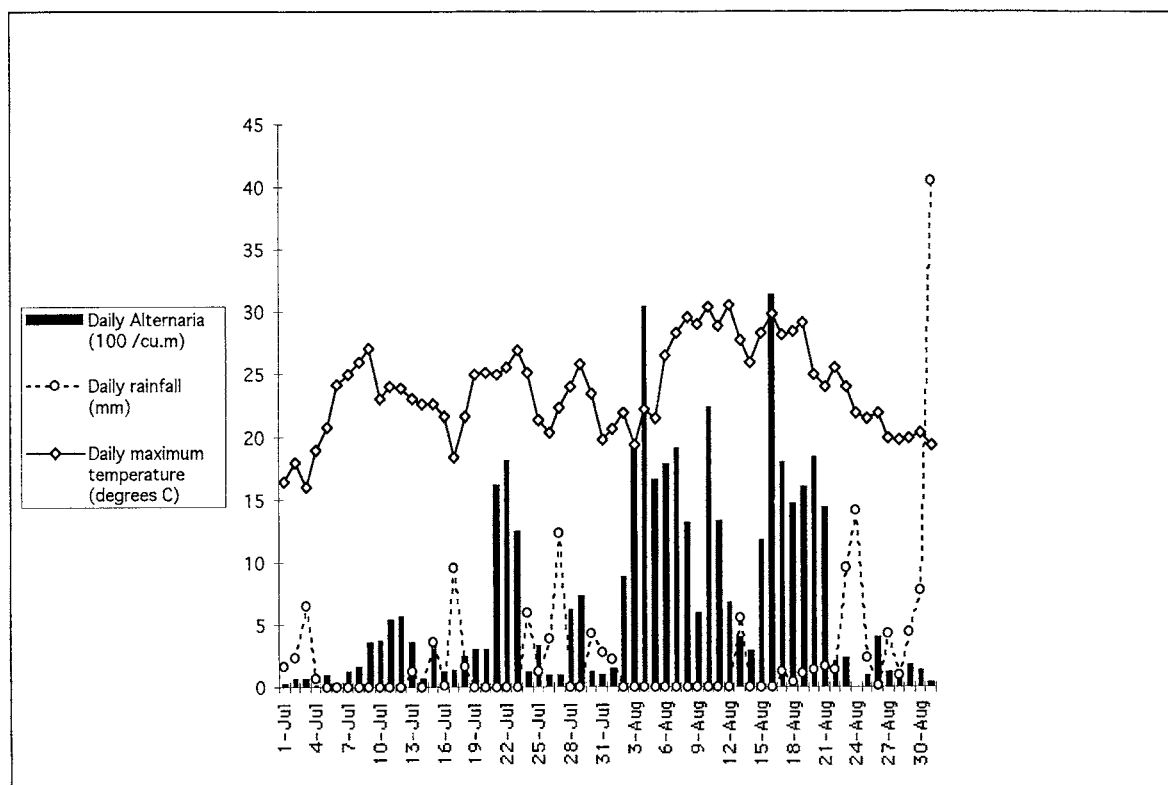


Figure 4. Daily July–August 1997 *Alternaria* spore concentrations compared with daily rainfall and maximum temperatures.

Table 2. *Alternaria*, rainfall and average temperatures in August 1991–1998.

Year	<i>Alternaria</i> August total (spores per cubic metre)	August rainfall in m.m.	Mean average daily August temperature °C
1991	9625	15	18
1992	16870	90	16
1993	4905	42	15
1994	6354	31	16
1995	9500	9	20
1996	15100	44	17
1997	32305	100	20
1998	9167	66	16

upward long term trend found in Derby *Alternaria* levels may result in an increase in patients with allergic symptoms. Previous work on birch (Emberlin et al., 1997) and grass pollen (Emberlin et al., 1999), has shown an increase in cumulative winter and early spring temperatures in Derby, and the *Alternaria* start

dates reflect this and show a trend to an earlier seasonal start already found in our pollen studies (Emberlin et al., 1997; Corden and Millington 1999; Corden et al., 2000).

When considering day to day Derby *Alternaria* spore levels, the high counts almost always occur on dry days and follow a similar diurnal rhythm to Derby grass pollen (Corden and Millington, 1991). The diurnal periodicity occurs slightly later in the day than the peak noon to afternoon diurnal periodicity found by Hirst in the UK (1953). We find daily *Alternaria* spore concentrations in Derby have a positive correlation with daily maximum temperatures as found in Spain (Fernández et al., 1998), whilst showing a slight negative correlation with daily precipitation, agreeing with work by Mitakakis et al. in Australia (1997). Gregory and Hirst (1957) found high *Alternaria* type spore concentrations most often in August, in warm dry spells after a period of wet weather. From the years 1991–1998 we have some evidence that although high daily *Alternaria* spore levels occur on dry days, or at least during dry weather, occasional rainfall results in higher monthly concentrations of *Alternaria* spores

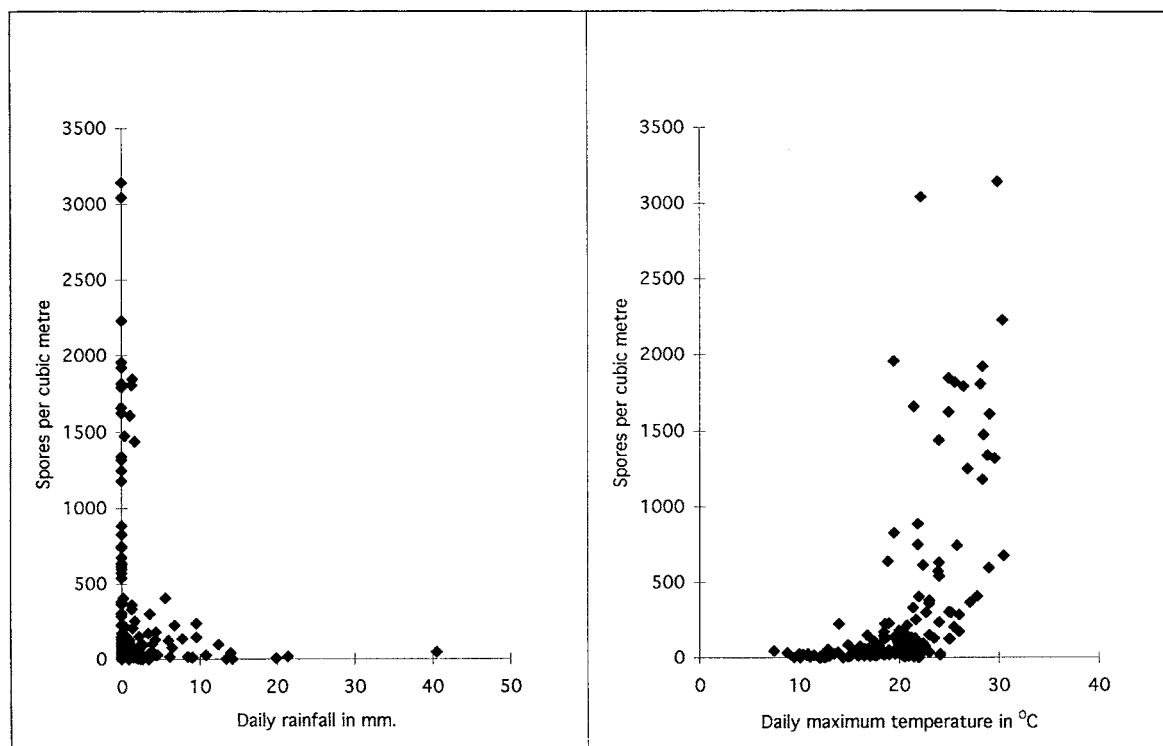


Figure 5. Scatter diagrams of the daily June–October 1997 *Alternaria* spore concentrations with daily rainfall and daily maximum temperatures.

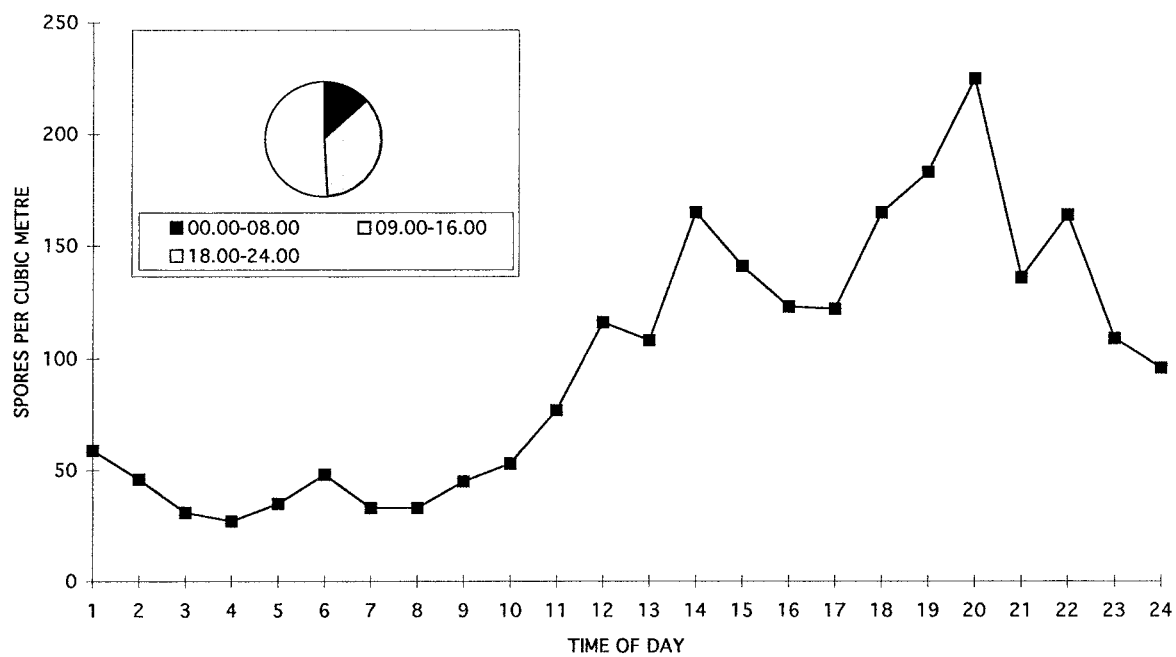


Figure 6. The diurnal periodicity of *Alternaria* spores, August 1986–1988.

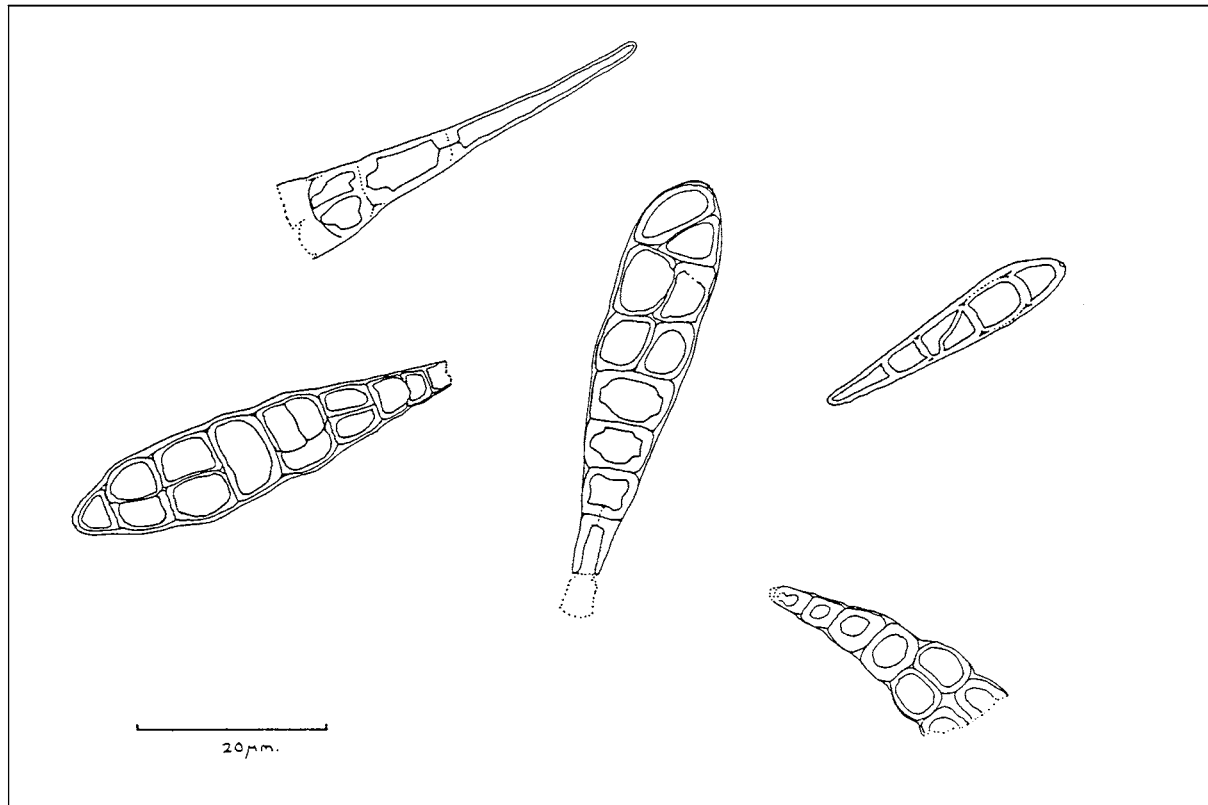


Figure 7. Scale drawing of *Alternaria* spores and fragments.

than during a very prolonged dry period. The multiple regression analysis of August 1991–1998 monthly data confirms the importance of rainfall and temperature on the August *Alternaria* totals. The very high August 1997 *Alternaria* spore levels were much higher than concentrations in August 1995, which was just as warm, but had only a fraction of the monthly rainfall.

In Derby the three main months for *Alternaria* spore release are July, August and September, which are the main months for combine harvesting. A study by Last (1955) found high concentrations of *Alternaria* spores within the crop of a wheat field, Sreeramulu (1958) described a very large increase (about 4 times) in the concentration of *Alternaria* spores associated with mowing grass and McCartney and Lacey (1992) found *Alternaria* spores were released from an oilseed rape crop in the daytime. We have long observed that bursts of *Alternaria* occur during periods of grass mowing, and at harvest time, especially when the weather has been warm and dry.

Alternaria is one of the most common atmospheric mould spores found in the USA with the greatest

numbers being found in grain growing areas such as the Midwest (O'Hollaren et al., 1991). Peat et al. (1995) found greater *Alternaria* sensitisation in children from the drier inland regions of Australia, whilst in rural Australian towns *Alternaria* spore levels were directly influenced by spore concentrations in the wheat crop outside the towns (Mitakakis et al., 1998). It is therefore likely that *Alternaria* spore concentrations in the city of Derby and surrounding areas will be greatly increased during harvesting. As *Alternaria* is a saprophyte living on most vegetation, the volumes of dust generated by combine harvesters will include many *Alternaria* spores which will be dispersed over a wide area. The increase in oil based crop planting results in a considerable extension of the harvesting season, as now not only is barley harvested in July in our area, but also winter rape at the end of that month. Wheat is harvested in August followed by spring rape in September and linseed in October/November. In 1942 Hyde and Williams (1946), were investigating *Alternaria* levels in Cardiff and they postulated that asthma attacks were more likely in the late summer

especially near wheat fields or wheat threshing. The present increase in harvesting duration and the milder weather in the autumn and winter may result in higher levels of *Alternaria* spores in the air and associated increases in asthma and rhinitis symptoms.

Frankland and Davies (1965) suggested that when the *Alternaria* count reaches 50 spores per cubic metre or above, patients sensitive to *Alternaria* develop symptoms. Every year in the Derby air there are a large number of days with over 50 *Alternaria* spores per cubic metre, with latterly an increasing number of days over 1000 spores per cubic metre. Targonski et al. (1995) suggested that the risk of asthma related deaths was increased 2.16 times when the total mould count was 1000 spores per cubic metre or above, therefore if daily levels of *Alternaria* spores are sometimes over 1000 then as *Alternaria* is known to be a high risk factor for severe asthma with resulting hospitalisation (O'Hollaren et al., 1991), our findings indicate that clinicians should consider *Alternaria* as a possible cause of severe asthma in periods of warm, dry summer weather, especially from June–September, and most particularly in August. Hjelmroos (1993) said *Alternaria* was under represented at roof level, so it is highly likely that people working at ground level especially close to crops must be exposed to far higher counts than are recorded at roof top height. The *Alternaria* spore is a large spore which is easy to identify, so perhaps daily spore counts should be made more readily available to clinicians and the general public in the UK, with routine monitoring of *Alternaria* at the same time as pollen being considered, as suggested by Bass and Morgan (1997) in Australia.

Licorish et al. (1985) suggested that with vertical presentation the spore had a diameter small enough to allow it to easily enter the lungs. We find evidence microscopically (Figure 7) of *Alternaria* spores broken into fragments with all or part of the distal portion sometimes separated from the rest of the spore. If these spores are broken up, as could easily happen during combining or mowing, then lung entry by spore fragments would be relatively easy.

Because *Alternaria* is found in the air at the same time as grass pollen and many more people are allergic to grass pollen, is the possibility of *Alternaria* being the culprit sometimes missed? A closer look at a patient's clinical history, or a rather longer period of seasonal symptoms, might indicate *Alternaria* sensitivity as suggested by Buisseret (1976). Fewer people are allergic to fungal spores but as mould spores have

been implicated as a major risk factor for fatal asthma attacks in Chicago USA (Targonoski et al., 1995) the increasing upward trend in Derby outdoor *Alternaria* spore levels should not be ignored.

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