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Incidence of *Alternaria* spores in the atmosphere of Murcia (SE Spain). Seasonal, monthly and intradiurnal variations

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SUMMARY

Variation in Alternaria *spores in the atmosphere of Murcia (SE Spain) were studied for 2 years. Using a Burkard volumetric sampler located about 19 m above ground level, spores were collected and counted hourly by light microscopy. The results suggest that species of* Alternaria *are present in the atmosphere of Murcia every day throughout the year. Mean daily concentration of* Alternaria *was 25 spores/m³. Seasonal variations showed the lowest concentrations during winter and the highest in autumn. In the monthly pattern two peaks were observed every year: the first in about May-June and the second occurring in October. In the intradiurnal variations, maximum concentrations were observed between 5:00-6:00 p.m. (Spanish official time). The variation of* Alternaria *spores in the atmosphere in Murcia seems to be related to different factors throughout the year.*

Key words: *Alternaria* - Aerobiology - Airborne spores - Seasonal variations - Monthly variations - Intradiurnal variations - Volumetric sampling

INTRODUCTION

Alternaria is a very common and well known genus of cosmopolitan fungus. It includes around 50 species of plant parasites, which provoke crop disease and great economic loss every year throughout the world, and saprophytes. Like other fungi, *Alternaria* disperses its numerous spores by releasing them into the turbulent airstreams of the atmosphere, allowing the spores to be distributed far from their source. This accounts for its high incidence in the air.

Along with *Cladosporium, Alternaria* has been considered to be the most prevalent of mould allergens (1, 2). It has been described as one of the major fungi responsible for inhalation allergies in man (3). In view of the fact that sporulation and dispersal depend on both biological and climatic processes, numerous efforts are made every year throughout the world to determine patterns and relationships between the airborne spores of *Alternaria* and meteorological conditions. The results of these investigations are usually of great interest, and combined with clinical data could improve diagnosis, treatment and prevention of respiratory allergic diseases.

Because of its geographical position in southeastern Spain, Murcia has a semiarid rainfall regime (250-300 mm), with a mean relative humidity of 58% and mean annual temperature of 18 °C. Within a radius of 15 km around the sampling site, a total of nearly 400,000 people live in about 50 towns and villages (including the city of Murcia). The incidence of *Alternaria* in the Murcia area accounts for 81% of the total allergenicity to fungi.

The aim of this study was to examine (on the basis of 2 years of data) variations in the annual, seasonal and hourly distributions of the airborne spores of *Alternaria* and find out their correlations with meteorological factors. Results might well be used in preventing diseases in conjunction with the previous knowledge about optimum growth temperatures and relative humidity ranges in the Murcia area (4).

MATERIAL AND METHODS

A Burkard volumetric 7-day recording spore trap (5) located at about 19 m above ground level on the ex-

posed flat roof of the Faculty of Veterinary Science, University of Murcia (110 m a.s.l., 4 km NW of the city of Murcia) was in operation from March 1, 1993 to February 28, 1995. There are no higher buildings in the immediate vicinity of this faculty. Daily slides from weekly ribbon strips were prepared following standard methods (6) and examined with light microscopy.

Hourly pollen data (Spanish official time, GMT +2 in spring-summer and GMT +1 in autumn-winter) were obtained by counting all *Alternaria* spores on four longitudinal transects. Missed and rainy days (those with rainfall above 0.1 l/m^3) were not taken into consideration for calculations.

Meteorological data were obtained from the Territorial Meteorology Center of the National Institute of Meteorology in Murcia (1 km from the sampling site). Because of the non-normal distribution of the *Alternaria* spore count, the relationships between conidia and weather variables were explored using the Spearman rank correlation coefficient method with the computer program SPSS release 5.0.1 (SPSS Inc., 1989-1992, Chicago). In graphical representations daily pollen counts (spores/m^3) were standardized into percentages to take into account the annual variations in pollen abundance.

RESULTS

Overall quantities of 9,740 and 5,743 spores/m^3 were obtained from March 1, 1993 to February 28, 1994 and from March 1, 1994 to February 28, 1995, respectively. The real content was probably higher because *Alternaria* is believed to be strongly underrepresented in the spore counts at roof level (7). The mean daily concentration varied between periods: 32 spores/m^3 for the first and 18 spores/m^3 for the second. A maximum (182 spores/m^3) was reached on June 10, 1993. For the whole period mean daily concentration was 25 spores/m^3. Although there were differences in *Alternaria* spore concentrations between the periods studied, annual distribution patterns were similar. Annual statistics are shown in Table 1.

For seasonal variation (Fig. 1), the highest concentration was seen in autumn (about 40% of the total). The lowest concentrations were registered in winter (about 7% to 8%). Summer presented higher concentrations than spring but both ranged from about 25% to 30%.

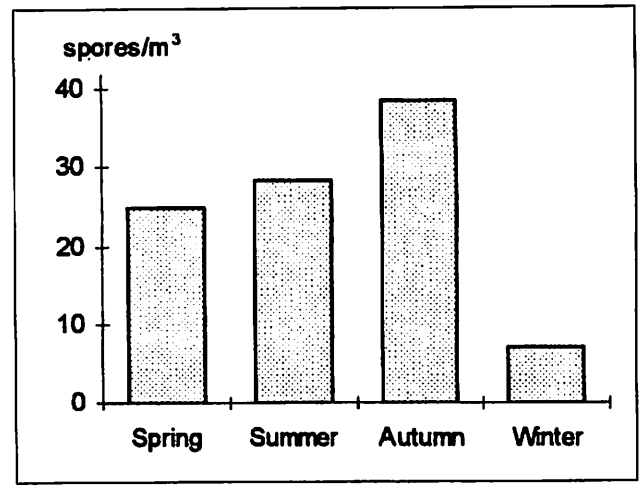

Fig. 1. Seasonal variations in the daily mean concentration of Alternaria *spores in Murcia.*

The patterns observed in the monthly variation (Fig. 2) were similar for both time periods (with lower levels for the second) and peaked twice each year: in May-June and in September-October, with a decrease in summer due to high temperatures and low relative humidity. Few *Alternaria* spores were found in winter, when mean minimum temperature was clearly below (2 to 3 °C) the optimum minimum (13 to 20 °C) proposed by

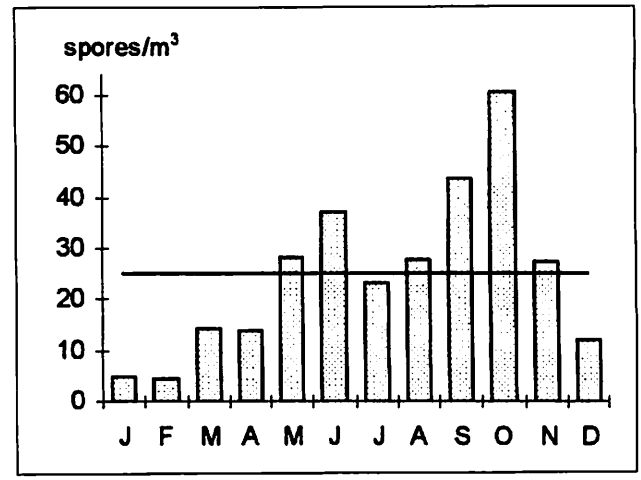

Fig. 2. Monthly variations in the daily mean concentration of Alternaria *spores in Murcia. Horizontal line indicates a mean daily average of 25 spores/m^3.*

Table 1
Annual statistics for *Alternaria* spores.

	Total count	Daily mean	SE	SD	Max.	Min.	Days
Whole period	15,483	24.5	1.0	26.2	182	0	633
1st period	9,740	31.9	1.7	30.3	182	2	305
2nd period	5,743	17.5	1.1	19.3	129	0	328

SE = standard error; SD = standard of deviation.

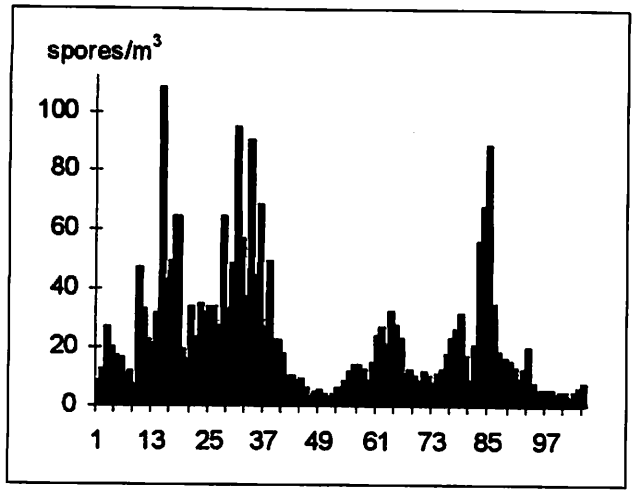

Fig. 3. Weekly variations of the daily mean concentration of Alternaria *spores in Murcia.*

Munuera *et al.* (4). Weekly pattern for *Alternaria* spores is presented in Fig. 3.

With each hourly count taken at the same time every day of the year, the mean number of *Alternaria* spores was calculated and an intradiurnal variation model was obtained (Fig. 4). Throughout the day, low concentrations were found during the night, with the minimum at about dawn and increasing constantly up to the maximum from 5:00 p.m. to 7:00 p.m. (Spanish official time).

Graphical and mathematical (Mann-Whitney U test) comparisons of the meteorological data showed differences between annual periods, with the second period being warmer and drier than the first, and the first period being windier than the second. However, annual tendencies in meteorological factors are both very similar.

Mathematical analyses showed very significant correlations ($p < 0.001$) between daily spore concentrations and some meteorological factors (Table 2): positive with spring and autumn temperatures; negative with air pressure in autumn; positive with evaporation except in winter; and positive with wind velocity during autumn. Other correlations ($p < 0.01$) were worth noting, including: positive with minimum temperature in winter; positive with wind direction in summer (SW), autumn (NE and SE) and winter (NE); negative with wind direction in spring (NW), summer (NE), autumn (SW) and winter (SW and NW). Correlations with whole periods are shown in Table 2.

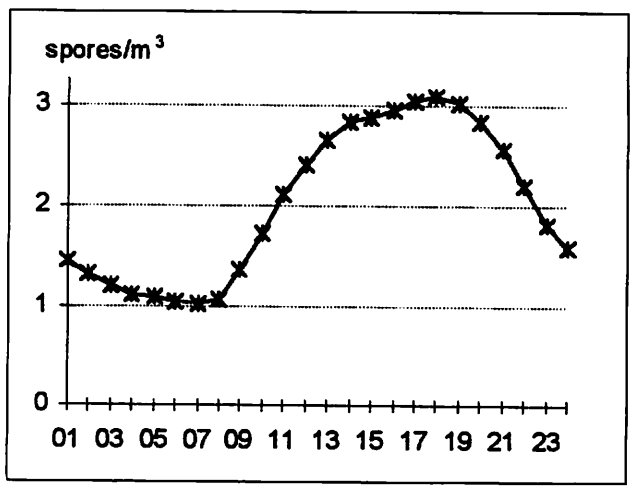

Fig. 4. Three-hour running mean (centered on the second hour) of the mean hourly average of Alternaria *concentrations in the atmosphere of Murcia.*

DISCUSSION

Comparison of airborne *Alternaria* spore concentration in two different time periods in Murcia indicated that the concentrations were not the same in both periods; however, considering the difference in weather conditions between the two periods, this was to be expected. Despite differences in concentration, trends of seasonal and monthly patterns were too similar for both periods. Noteworthy differences were not found in daily variation patterns obtained for every month and season, indicating that *Alternaria* releases its spores in a constant daily pattern throughout the year. Total daily concentration depended on the time of the year.

Overall quantities of *Alternaria* spores in the atmosphere in Murcia were higher than those reported in other countries: 3 to 6 times higher than in Lucknow (India) (8), 2 to 14 times higher than in Stockholm (Sweden) (7), 2 to 7 times higher than in Cagliari (Italy) (9) and 1 to 2 times higher than in Melbourne (Australia) (10).

The annual peaks observed (the first in early summer and the second in autumn) were consistent with those obtained in other cities with a similar climate, such as Cagliari (9), Melbourne (10) and Brisbane (Australia) (11). In Cagliari (Italy), *Alternaria* peaks from late April to July and in autumn. More similar patterns to those obtained in Murcia were recorded in Melbourne and Brisbane (Australia), with peaks in early summer followed by a second seasonal peak in autumn (10, 11). In all cases, the peaks reverse their relative significance in comparison with Murcia, where highest concentrations occurred during autumn rather than summer.

Intradiurnal pattern of spore variation in Murcia (Fig. 4) supports the idea that *Alternaria* is a late afternoon taxon rather than an early afternoon one as proposed by Gregory (12) and Srivastava and Wadhwani (8). Considering correlations between *Alternaria* spore concentrations and wind direction (Table 2), and keeping in mind that occurrence curves are broader and lower and delaying peaks are a consequence of remote pollen sources (13-15), high *Alternaria* spore concentrations in the late afternoon in Murcia could be due to long distance transport and subsequent redepositing in the sampling site area.

In relation to exposure levels and sensitization and exacerbation of allergenic symptoms in Murcia, no data

Table 2
Spearman correlation coefficients between daily *Alternaria* spore concentrations and weather variables.

		Whole period	1st period	2nd period	Spring	Summer	Autumn	Winter
Wind	Direction	−0.2164***	−0.2588***	−0.2052***	−0.1687*	0.1098	−0.1528*	−0.2362**
	Velocity	0.0435	0.0484	0.0561	−0.0925	−0.004	0.2944***	0.0724
	Run	0.0084	0.0149	0.0421	−0.0735	−0.2201**	0.2127**	−0.0046
	Calms	0.0539	−0.1002	0.1347*	0.1728*	−0.0073	−0.2071**	0.0992
	NE	0.2428***	0.2840***	0.2474***	0.1022	−0.2146**	0.2341**	0.2291**
	SE	0.1682***	0.2444***	0.1826***	0.092	−0.1087	0.2240**	−0.1213
	SW	−0.1737***	−0.1776***	−0.1532**	−0.0467	0.1863*	−0.2097**	−0.2314**
	NW	−0.1887***	−0.1859***	−0.1734***	−0.2273**	0.1198	−0.0345	−0.1644**
Air pressure	Maximum	−0.3661***	−0.3052***	−0.4161***	−0.0168	−0.1183	−0.4940***	−0.0158
	Minimum	−0.2811***	−0.1849***	−0.3612***	0.0182	−0.0924	−0.4446***	−0.0016
Tempera-	Maximum	0.4633***	0.5468***	0.4843***	0.5093***	−0.1245	0.4255***	0.083
ture	Minimum	0.5257***	0.6214***	0.5179***	0.4422***	−0.1879*	0.4614***	0.1892*
	Min. 15 cm a.g.l.	0.5264***	0.6253***	0.4973***	0.4228***	−0.1192	0.4296***	0.1838*
	Mean	0.5175***	0.6088***	0.5307***	0.5184***	−0.1812*	0.5060***	0.1466
Others	Rainfall	0.0178	−0.0412	0.0523	−0.1888*	0.1027	0.0227	0.0565
	Evaporation	0.3257***	0.3738***	0.2261***	0.3074***	0.3079***	0.4074***	0.1375
	Relative humidity	−0.0655	−0.0419	−0.0816	−0.0490	−0.0852	−0.1188	0.0996
	Insolation	−0.1087**	−0.0944	−0.1107*	0.2159**	−0.1176	−0.2033**	−0.0941

*p <0.05, **p <0.01, ***p <0.001.

are available at the moment, but some studies are now underway. We hope that results will be able to be presented in a few months.

Relationships between airborne *Alternaria* spores and weather variables (Table 2) only partly agree with those found out in other localities. Positive correlations with winter relative humidity and temperature were reported by Rutherford *et al.* (11) in Brisbane (Australia) as well as by Palmas and Cosentino (16) in Cagliari (Italy), but in this case the relation was negative with relative humidity. In Melbourne (Australia) Mitakakis *et al.* (10) reported negative correlation with rainfall and positive with mean temperature. In Stockholm (Sweden), Hjelmroos (7) noticed positive correlations with relative humidity, temperature, total cloud, air pressure, wind velocity and wind direction depending on the year.

Finally, wind direction showed negative correlations with *Alternaria* spore concentrations except in summer. This was only a consequence of the positive relationship with eastern winds (just from 0° to 180°) and the negative one with western winds (from 180° to 360°), with the highest spore counts being linked to northeastern winds (from 0° to 90°). In summer, the highest spore counts seem to be related with western winds (from 180° to 360°), reversing tendencies in comparison with the rest of the year. In Murcia, correlations were similar throughout the two periods, showing different values for every season.

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RESUMEN

Durante 2 años se ha estudiado la variación en las cantidades de esporas de Alternaria *presentes en la atmósfera de la ciudad de Murcia (SE España). Se ha usado un captador volumétrico Burkard situado a 19 m de altura para recolectar las esporas, y microscopía óptica para su identificación y recuento. Las esporas de* Alternaria *están presentes en la atmósfera de Murcia durante todo el año, con una concentración media de 25 esporas/m³. Las menores concentraciones se registran en invierno y las mayores en otoño. Se observaron dos máximos a lo largo del año, el primero en mayo-junio y el segundo en octubre. A lo largo del día se notó un progresivo aumento de las concentraciones desde primeras horas del día (8-9 h), hasta alcanzar un máximo al final de la tarde (17-19 h). La presencia de esporas de* Alternaria *en la atmósfera de Murcia parece estar relacionada con distintos parámetros meteorológicos según el momento del año.*

Palabras clave: *Alternaria* - Aerobiología - Esporas aerovagantes - Variación estacional - Variación mensual - Variación intradiurna - Muestreo volumétrico

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