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Airborne *Alternaria* spores in SE Spain (1993–98)

Occurrence patterns, relationship with weather variables and prediction models

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The annual, seasonal and hourly distribution of *Alternaria* spores in the air of Murcia, SE España, was studied on a six-year period. The relationships between *Alternaria* spore concentrations and meteorological factors were investigated.

Alternaria is a late afternoon taxon in the Murcia city, with maximum spore concentrations occurring between 13.00 h and 21.00 h. *Alternaria* spores are present in the atmosphere throughout the year, with a main spore season extending from March to October, and showing two peaks as a consequence of the summer drop in concentration.

Alternaria spore concentrations correlate well with Poaceae and Chenopodiaceae pollen counts, suggesting these plants could be important hosts, but not the only ones, because many crops are growing just when peaks occur. Low wind velocities favoured high spore counts. Correlation with temperature was positive in five of the six years. Mean temperature is the factor which best explain spore levels. The best prediction model obtained explains 74% of the observed variance in *Alternaria* levels (in a five steps scale) by using mean temperature alone.

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Murcia is a predominantly agricultural region in the south-eastern Spain. Non-irrigated (vine, almond trees, cereals) and irrigated (vine, citrus-trees, fruit-trees, greens and vegetables, alfalfa, cotton) crops are growing throughout the year because of the Mediterranean climate (dry hot summers, mild winters and maximum rainfall in spring and autumn; average annual rainfall = 250–300 mm; mean relative humidity = 58%; mean annual temperature = 16.8 °C). Natural vegetation consists of woodland and brushwood. Woodlands are found on mountainous areas and are dominated by *Pinus halepensis*, but frequently included evergreen oaks as undergrowth or as small trees. Most of the forested and brushwood areas have a ground covered of *Brachypodium*. Bushy areas are heterogeneous and widespread consisting of rosemary (*Rosmarinus officinalis*), thyme (*Thymus* sp.), esparto (*Stipa tenacissima*) and *Anthyllis cytisoides* communities, which include several species of Poaceae, *Cistus*, *Helianthemum*, *Fumana*, *Sideritis*, and *Teucrium*. Those communities dominated by *Lygeum spartum* include *Salsola genistoides* and *Limonium* sp. In uncultivated areas, there are very frequent nitrophilous communities rich in Chenopodiaceae, Poaceae, Brassicaceae, *Artemisia* and other Asteraceae, *Parietaria*, and other herbs. *Artemisia* occurs even together with Chenopodiaceae and *Lygeum spartum*, in salty soils.

Alternaria is an ubiquitous and well-known genus with ca. 50 saprotrophic or plant pathogenic species that cause much economic loss throughout the world. Its spores are air spread, and they are estimated to be strongly underrepresented at roof level because their relatively large size (Hjelmroos 1993).

Alternaria, along with *Cladosporium*, is considered to be the most prevalent of mould allergens (Budd 1986, Vijay et al. 1991) and have been described as one of the main fungi responsible for inhalation allergies in humans (Caretta 1992). In Murcia, 81% of the fungal-allergic population is affected by *Alternaria* (Pagán et al. 1984). Knowledge of airborne spore concentrations and their relationship with weather variables is of great importance in the management and/or prevention of respiratory allergic diseases (Hasnain 1993) since, combined with clinical data, it can improve diagnosis, treatment and prevention. On the other hand, since relationships between airborne spore concentrations and plant diseases have been found (Eversmeyer & Kramer 1992, Picco 1992), knowledge of airborne plant pathogen spore trends can also be used in disease treatment, for example in the early detection of infections (Bugiani & Govoni 1991, Ponti & Cavanni 1992, Roses-Codinachs et al. 1992, Smith et al. 1992), or by allowing more efficient and reliable use of pesticides (Bugiani et al. 1996, Montesinos et al. 1995).

The aim of this work was to study variations in the annual, seasonal and hourly distributions of *Alternaria* airborne spores in Murcia, and to investigate relationships between concentrations of *Alternaria* spores and meteorological factors.

MATERIAL AND METHODS

From 1 March 1993 to 31 December 1998 a volumetric seven-day recording spore trap (type Hirst; manufactured by Burkard) located at about 19 m a.g.l. was operated on the exposed flat roof of the Veterinary Faculty of Murcia University (110 m a.s.l., 38°01'N, 01°10'W), 4 km NW of Murcia city centre (Fig. 1). There were no higher buildings in the immediate vicinity. Daily slides were prepared and subsequently examined with light microscopy following standard methods of the Spanish Aerobiology Network (Domínguez et al. 1991).

The daily meteorological data used in this study were obtained from the main meteorological station of the Instituto Nacional de Meteorología in Murcia, located about 2.3 km SW of the sampling site in the Centro Meteorológico Territorial (38°00'10"N, 01°10'10"W, 62 m a.s.l.). The surrounding area consists of a marly soil with low-density plant cover dominated by Chenopodiaceae, Brassicaceae and grasses. Only a few small houses are found near the eastern side of the meteorological centre, thus the site should be more representative of open country than a built urban area.

Hourly spore concentrations (Spanish official time, GMT +2 in spring-summer and GMT +1 in autumn-winter) were obtained by counting (at ×400) all *Alternaria* spores on 4 longitudinal transects. The main characteristics of *Alternaria* spore record for six years is presented in Table I. Those daily slides spoiled by insects, lost by spore trap failures or corresponding to rainy days (those with rainfall above 1 mm/day) were not taken into account in the statistical analyses. Thus, 76%–89% of the daily spore concentrations was considered. To obtain hourly curves (Fig. 5) only rainless days having overmean spore concentrations (26%–34% of the annual number of days) were considered.

Because of the non-normal distribution of *Alternaria* spore counts, even after logarithmic or square root transformation, non-parametric tests were used to explore relationships between conidia concentrations and weather variables. All calculations were made using SPSS 9.0.1 (SPSS Inc., 1989–1999, Chicago).

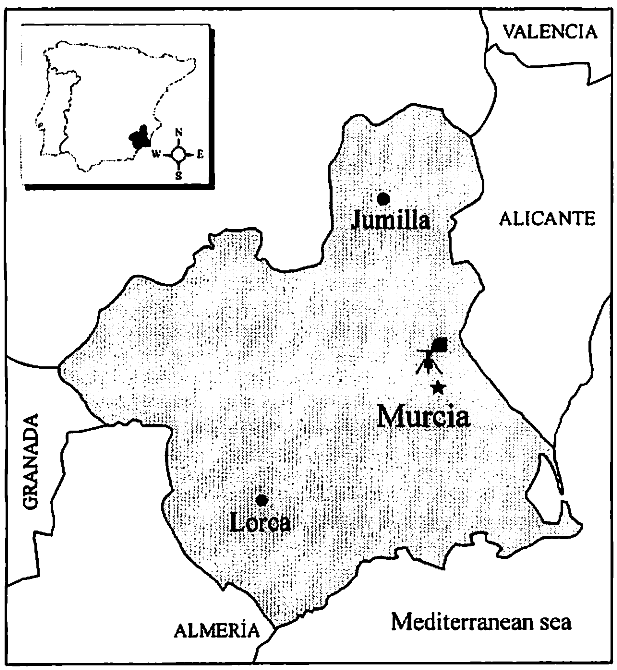

Fig. 1. Location map.

RESULTS

Seasonal variation

Alternaria spores were present in the atmosphere of Murcia throughout the year (Fig. 2) with daily mean concentrations ranging from 22 to 50 spores m^{-3}. The main spore season, defined as the period between the date at which the sum of daily mean concentrations reached 5% of the total sum, and the time when the sum reached 95% (Nilsson & Persson 1981), extended from early March to early October. The average length for the spore season was 264 days, varying between 303 in 1996 and 244 in 1997 (Table I). In 1993 the main spore season could be artificially short (237 days) because sampling did not begin until 1 March. In the main spore season the mean daily concentration (excluding missing and rainy days) for the six years was 43 spores m^{-3}. There were two peaks of spore concentration, one in May–June, and one in September–October (Fig. 3). The maximum daily average concentration (366 spores m^{-3}) was recorded on 5 October 1997.

A Kruskal-Wallis test was used to explore differences between spore concentrations for each of the six years. Results indicate that the spore samples in main spore seasons consist of at least two different "statistical populations", probably due to climate and related changes rather than in year-to-year differences in host plants populations. After a pairwise multiple comparison, the six seasons were divided into three homogeneous groups (Table II).

For the whole year, *Alternaria* conidia concentration was weakly correlated with Poaceae (Spearman correlation coefficient = 0.237, P = 0.001) and Chenopodiaceae (Spearman correlation coefficient = 0.412, P = 0.001) pollen concentration. Spore concentrations from mid February (when spring starts in Murcia) to mid May coincide with changes in Poaceae and Chenopodiaceae pollen concentrations (Fig. 4). Later, *Alternaria* spore concentrations continue increasing when pollen counts drop. In late summer, from mid August on, *Alternaria* correlate again with Chenopodiaceae pollen for 5–6 weeks, just before the annual maximum for *Alternaria* is reached in late September.

Hourly variation

Distribution of spore concentrations through the day was similar for the six studied years (Fig. 5). Concentrations were lowest in the early morning (about 08.00 h), increased quickly until ca 14.00 h, followed by a slight rise to a maximum at about 19.00 h, and then decreased steadily until the early morning minimum. Patterns for 1994 and 1998 show slight, but non-significant, differences from the mean curve and the other years.

Correlations with weather variables

Three temperature parameters consistently correlate with *Alternaria* spore concentrations (Table III), suggesting a significant and positive effect of temperature on *Alternaria* spore concentration. Correlations with other meteorological parameters were not consistent in significance and even in sign between years. Perhaps should be mentioned the mainly

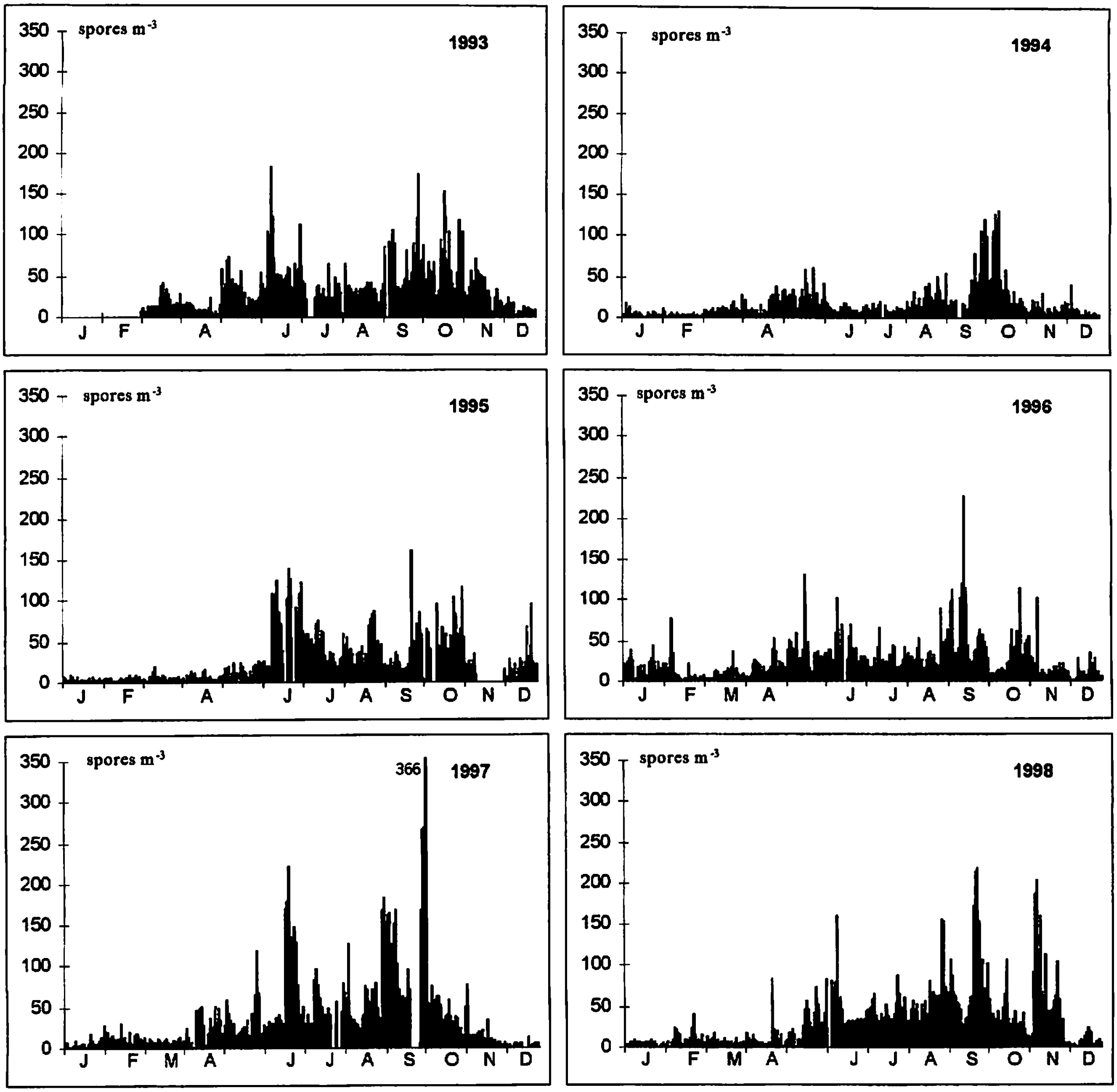

Fig. 2. Daily mean spore concentrations of *Alternaria* in Murcia for 1993–98. Sampling start on 1 March 1993. Because 31-day units have been considered to divide year in months, some differences can be observed between dates showed in Table I and supposed dates from graphs. Blanks correspond to non-data days.

positive effect that evaporation seems to have over *Alternaria* spore concentrations and the negative one of NW winds.

Regression analysis

Linear stepwise regression analysis (Table IV), showed that wind from first quadrant and relative humidity, together with minimum temperature, were the meteorological factors which best explain variations in airborne spore concentrations of *Alternaria* in Murcia but, having into consideration that only 13% of the variance is explained, model is not satisfactory. When the day-before spore concentration is used as an additional independent variable, is introduced as the first step equation and it can explain 59.8% of the observed variance by itself (Table V), and with maximum temperature and relative humidity explains almost 61%. Those data come to confirm that, in Murcia, actual-day weather is not affecting *Alternaria* spore concentrations in a significant way, and only provide prediction models associated to great errors.

When looking for useful prediction models we are really more interested in good approximation to levels (with low error) rather than in exact prediction of spore number (associated with great error). So, and having in mind that

Table I. *Main characteristics of Alternaria spore records.*

* In brackets rainless and overmean concentration days; ** total concentration in spores m^{-3} for the referred period; DFS = Days from start – Mean data for the main spore season have been calculated omitting 1993, when sampling started 1 March.

	Main spore season				1st peak day			2nd peak day			Annual
	Start	End	Days*	Conc.**	Date	DFS	Conc.**	Date	DFS	Conc.**	Conc.**
1993	1-Apr	24-Nov	237 (196/71)	>9592	10-June	70	184	2-Oct	184	176	>10628
1994	4-Mar	1-Dec	272 (243/77)	5682	26-May	83	61	14-Oct	224	131	6305
1995	28-Mar	20-Dec	267 (202/75)	8157	14-June	78	141	25-Sep	181	162	9051
1996	26-Jan	24-Nov	303 (250/84)	8569	17-May	112	132	16-Sep	234	228	9517
1997	4-Mar	3-Nov	244 (201/63)	11254	22-June	110	222	5-Oct	215	366	12498
1998	9-Mar	24-Nov	260 (227/88)	11028	11-June	94	160	26-Sep	201	219	12225
Mean	2-Mar	26-Nov	269 (224/77)	8938	6-June	96	150	29-Sep	211	214	10037

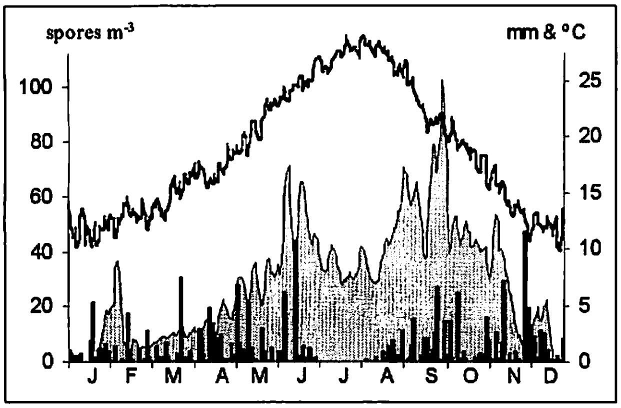

Fig. 3. Five days running means of the daily average spore concentrations of *Alternaria* during the six main spore seasons (shadowed area), average mean temperature (black line) and average rainfall (columns).

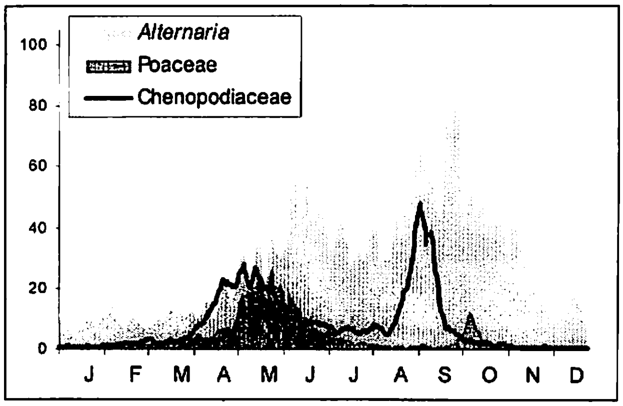

Fig. 4. Daily average of *Alternaria* conidia and Poaceae and Chenopodiaceae pollen concentrations (particles $m^{-3} d^{-1}$) in Murcia atmosphere.

Table II. *Duncan's test for the main spore seasons of Alternaria.*

Group 1		Group 2		Group 3	
Year	Mean	Year	Mean	Year	Mean
1994	21.3	1996	30.6	1993	44.4
		1995	36.3	1998	44.8
				1997	49.7

Alternaria airborne spore concentrations correlate well with temperatures, we tried a third method (curvefit regression analysis) where, in order to minimise the considerable day to day fluctuation observed, *Alternaria* spore concentrations were categorised into five classes or levels: VL (very low: 0–15 spores m^{-3}), L (low: 16–30 spores m^{-3}), M (medium: 31–70 spores m^{-3}), H (high: 71–120 spores m^{-3}) and VH (very high: >121 spores m^{-3}). Then the average level was calculated for every temperature and computed in order to obtain a formula explaining distribution of average level of *Alternaria* spores in relation with temperature. The best result was obtained for the cubic polynomial using mean temperature as the independent variable to predict current day concentration level (Table VI, Fig. 6) and the equation explains more than 73% of the variance observed in the average level corresponding to every mean temperature and give us the correct level in 76%, overestimation in 12% and underestimation in 12% of the cases:

Tentative prediction model 3 (Table VI)

$$y = -0.4826\, T_{mean} + 0.0375\, T^2_{mean} - 0.0007\, T^3_{mean}3 + 2.8554$$

where, y = expected level of *Alternaria* concentration

Comparing the expected level of *Alternaria* conidia in the atmosphere with the actual one in the day-to-day data set, the formula give us the correct level in 39% of the cases, a higher level in 36% of the days and an underestimation 25% of the times. When using actual spore counts (not categorised into five levels) to generate a similar equation, we can explain 43.4% of the variance, and an optimum mean temperature (concentrations over 45 spore m^{-3} d^{-1}) was observed between 22 and 28 °C. Maximum temperatures over 33 °C clearly provoke falls in spore concentration, maybe because the effect of temperature on other factors affecting spore production and release. Meteorological variables other than temperatures were tested, but no valid equations were generated.

DISCUSSION

Seasonal variation patterns (Figs 2, 3) confirm preliminary results presented after a 2-year study (Munuera et al. 1998), but the six year mean daily average for the main spore

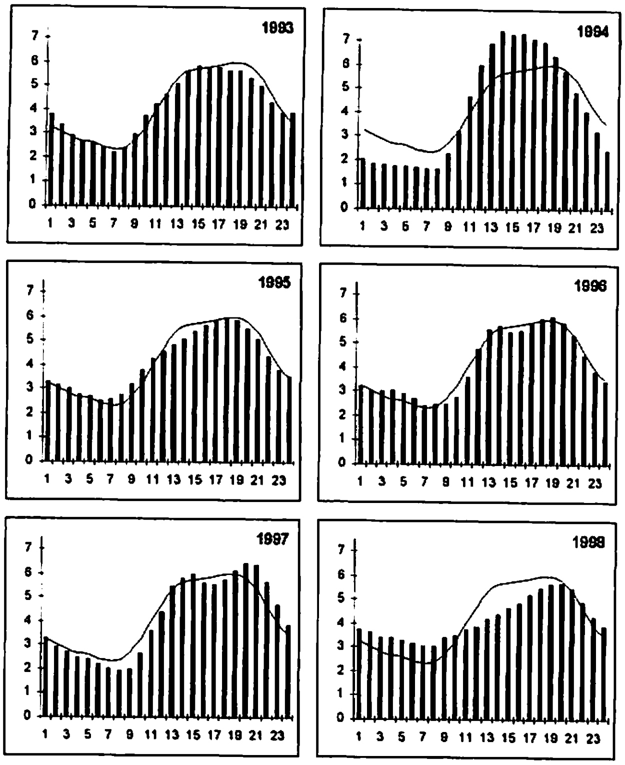

Fig. 5. Three hours running means of hourly percentages of daily airborne spore counts of *Alternaria*. Curve shows the mean for 1993–98.

Table III. *Correlations between temperatures and spore concentrations during the main spore seasons of Alternaria for the studied years and for the whole period.*

2-tailed Spearman correlation coefficients. Signification level: [a]0.1 [b]0. 05 [c]0.01.

	1993	1994	1995	1996	1997	1998	1993–98
T_{max}	0.130[a]	0.172[c]	0.454[c]	0.461[c]	0.513[c]	0.431[c]	0.345[c]
T_{mean}	0.129[a]	0.197[c]	0.550[c]	0.458[c]	0.562[c]	0.470[c]	0.385[c]
T_{min}	0.131[a]	0.179[c]	0.580[c]	0.444[c]	0.563[c]	0.465[c]	0.395[c]

season (excluding missing and rainy days) was higher than formerly reported (43 instead of 25 spores m^{-3} d^{-1}). Unlike in northern España (Herrero & Zaldivar 1997) and for temperate zones above 1000 m a.s.l. (Ebner et al. 1989), there was a notable decline in spore concentrations in mid-summer, as also seen in Melbourne (Mitakakis et al. 1997) and Sydney (Bass & Morgan 1997), when mean temperature is supposed to be optimum for growth. This fall could be a consequence of winds coming mainly from the NE, with less cultivation, but may be related to (i) dryness, with mean daily precipitation in July/August in the years studied of 0.012–0.18 mm instead of the normal 5–10 mm (Murcian long-term mean 1961–90), and (ii) high maximum temepratures in July of 31.7–35.7 °C (mean 34.6 °C) and August 33–35.9 °C (mean 34.9 °C), compared to long-term means of 25.3 °C (July) and 28.7 °C (August). These temperatures are much higher than in northern Spain and temperate zones, and clearly over the maximum temperature of 34 °C above which spore concentrations begin to fall (Munuera & Carrión 1995). These meteorological conditions could have a direct effect on spore production, or indirectly affect it by its effect on substrates colonised by *Alternaria*. As *Alternaria* sporulates better in darkness than in full light (Srivastava & Wadhwani 1992), and is negatively affected by cloudiness less than 40% (Hjelmroos 1993), the high summer levels of sunshine and high insolation (that is, low cloudiness) in Murcia area could be an influence, even when no correlations where found between *Alternaria* spore counts and insolation.

From mid winter on, increases in *Alternaria* spore concentrations seem to be related with Poaceae and Chenopodiaceae (Fig. 4), but in the beginning, it must be not as dead growth substratum but as hosts. Although there must not be the only substratum, because mid winter is the time when "spring" stars and annual plants and crops are growing in the Murcia area. A peak occurring in June could corresponds, not only to the colonisation of dead annual grasses, as proposed for Melbourne (Australia) by Mitakakis et al. (1997), but to the colonisation of dead tissues of other widespread annual plants such as Brassicaceae, Chenopodiaceae, Plantaginaceae, among others. Bass & Morgan (1997) reported a positive correlation between Poaceae pollen and *Alternaria* spores to Sydney. Cotton fields have been associated with high and continuous *Alternaria* spore levels in Australia (Bass & Morgan 1997). These crops are common in Vega del Guadalentín, SW of the sampling site, and may be associated with this autumn peak too. The source of *Alternaria* spores in October–November is unclear, but may be leaf trees, falling at this time after a long senescence period.

The hourly pattern of spore concentrations found here agrees with that previously reported for Murcia (Munuera et al. 1998) and confirm that in Murcia *Alternaria* is a late afternoon taxon (maximum from 13.00 h to 21.00 h) rather than a morning-early afternoon one (maximum from 08.00 h to 17.00 h) as proposed by Gregory (1973) and Srivastava & Wadhwani (1992). This late afternoon maximum could be related to: (i) a real late afternoon release of conidia from local sources, or (ii) a delay in sampling of spores released earlier and being transported to the sampler from rural areas (15–30 km away).

Although *Alternaria* is believed to be favoured by relatively strong winds (Hjelmroos 1993), in this study the positive and significant correlation with wind velocity was only for 1997. In addition, there were mainly positive correlations with calms, being significant for 1996, 1998 and the whole period. Notwithstanding, in other sites e.g. Cádiz, España (González Minero et al. 1994), Brisbane, Australia (Rutherford et al. 1997), Stockholm, Sweden (Hjelmroos 1993), Cape Town, Pretoria and Johannesburg, South Africa (Cadman 1991, Hawke & Meadows 1989), and Auckland, New Zealand (Hasnain 1993), positive correlations with wind velocity have been described. Positive correlations with temperature similar to those observed in Murcia and shown in Table III have been reported extensively in other sites throughout the world (Fernández-González et al. 1993, Hjelmroos 1993, Lyon et al. 1984, Mitakakis et al. 1997, Palmas & Cosentino 1990, Paredes et al. 1997, Rosas et al. 1990, Srivastava & Wadhwani 1992).

Table IV. *Stepwise linear regression analysis for Alternaria spore concentrations during the whole main spore season (6 years).* Wind from first quadrant (W_1) and relative humidity (RH) are used as independent variables.
* In a similar model obtained by using only data for the named year.

Summary	
Data	1399
Multiple R	0.362
R^2	0.131
Adjusted R^2	0.129
Standard error	32.38

Regression coefficients				
Variable	Coefficient	Sum of squares	T	P
T_{min}	2.575	0.190	13.545	0.000
W_1	−1.410	0.182	−7.765	0.000
HR	0.491	0.074	6.631	0.000
Constant	−16.219	4.857	−3.340	0.001

Explained percentage	
Step	Adjusted R^2
1	0.078
2	0.101
3	0.129

Analysis of variance					
	DF	Sum of squares	Mean square	F	P
Regression	3	208070.389	69356.796	66.115	0.000
Residual	1312	1376334.876	1049.036		

	1993	1994	1995	1996	1997	1998
Percentage variance explained*	9.0	8.6	21.9	11.1	20.7	31.1
Significance of the model (P value)	0.001	0.001	0.001	0.001	0.001	0.001

Table V. *Stepwise linear regression analysis for Alternaria spore concentrations during the whole main spore season (6 years).* Day-before spore concentrations (S_p), maximum temperature (T_{max}) and relative humidity (RH) are used as independent variables.
* In a similar model obtained by using only data for the named year.

Summary	
Data	1399
Multiple R	0.780
R^2	0.608
Adjusted R^2	0.607
Standard error	21.8346

Regression coefficients				
Variable	Coefficient	Sum of squares	T	P
S_p	0.739	0.018	41.883	0.000
T_{max}	0.651	0.121	5.373	0.000
HR	0.189	0.051	3.709	0.000
Constant	−18.277	4.909	−3.723	0.000

Explained percentage	
Step	Adjusted R^2
1	0.598
2	0.603
3	0.607

Analysis of variance					
	DF	Sum of squares	Mean square	F	P
Regression	3	948800.705	316266.902	663.381	0.000
Residual	1282	611193.952	476.750		

	1993	1994	1995	1996	1997	1998
Percentage variance explained*	53.1	65.1	68.2	53.5	54.7	64.3
Significance of the model (P value)	0.001	0.001	0.001	0.001	0.001	0.001

We consider, tentative prediction model for *Alternaria* spore levels here presented (Table VI) is realistic because, even when it does not permit to "know exact number" of spores, it give us useful information about level would be reached, and only mean temperature is needed. Error is acceptable because the model underestimates (saying don't worry when maybe you have to do) only in 12% of predictions.

The optimum mean daily temperature for high (>45 spores m^{-3} d^{-1}) *Alternaria* spore concentrations in Murcia, 22–28 °C, is similar to that found by Srivastava & Wadhwani (1992) in India, and coincides with that found by Hjelmroos (1993) in Stockholm and is 2 °C higher than the optimum found for Murcia in a 1-year study (Munuera & Carrión 1995).

CONCLUSIONS

Alternaria spores occur in Murcia atmosphere every day, with a mean of 43 spores m^{-3} d^{-1}. The main spore season

Table VI. *Stepwise linear regression analysis for Alternaria spore concentrations during the whole main spore season (6 years).*
Day-before spore concentrations (S_p), maximum temperature (T_{max}) and relative humidity (RH) are used as independent variables.
* In a similar model obtained by using only data for the named year.

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T_{max}	0.651	0.121	5.373	0.000
HR	0.189	0.051	3.709	0.000
Constant	−18.277	4.909	−3.723	0.000

Explained percentage

Step	Adjusted R^2
1	0.598
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Analysis of variance

	DF	Sum of squares	Mean square	F	P
Regression	3	948800.705	316266.902	663.381	0.000
Residual	1282	611193.952	476.750		

	1993	1994	1995	1996	1997	1998
Percentage variance explained*	53.1	65.1	68.2	53.5	54.7	64.3
Significance of the model (P value)	0.001	0.001	0.001	0.001	0.001	0.001

Table VII. *Cubic regression analysis for Alternaria mean spore concentration levels observed for every mean temperature (T_{mean}) during the whole main spore season (6 years).*
Day-before spore concentrations (S_p), maximum temperature (T_{max}) and relative humidity (RH) are used as independent variables.
* In a similar model obtained by using only data for the named year.

Summary	
Data	252
Multiple R	0.860
R^2	0.740
Adjusted R^2	0.736
Standard error	0.392

Regression coefficients				
Variable	Coefficient	Sum of squares	T	P
T_{mean}	−0.4826	0.0656	−7.352	0.000
T^2_{mean}	0.0375	0.0037	10.080	0.000
T^3_{mean}	−0.0007	6.51E-05	−11.266	0.000
Constant	2.8554	0.3493	8.175	0.000

Analysis of variance

	DF	Sum of squares	Mean square	F	P
Regression	3	108.724	36.242	235.804	0.000
Residual	249	38.269	0.154		

	1993	1994	1995	1996	1997	1998
Percentage variance explained*	20.8	19.4	39.6	32.9	52.7	47.4
Significance of the model (P value)	0.000	0.000	0.000	0.000	0.000	0.000

last from early March to early October (mean of 264 days). Two peaks occur, one in the spring and one in the autumn. The mid-summer drop in concentrations maybe is a consequence of dry weather and high maximum temperatures that occur during July and August in Murcia.

The aerobiological data suggest that Poaceae and Chenopodiaceae may host a source of *Alternaria*, but annual herbs (Brassicaceae, Plantaginaceae) maybe sources for the early-summer peak. The late-summer peak may be due to Chenopodiaceae flowers acting as hosts and later with diverse sources such as cotton fields and falling leafs.

In Murcia area, *Alternaria* spores behave as late afternoon taxa rather than a morning one as found in other localities. This may not be a real difference for *Alternaria* spores in Murcia, but caused by transport of spores from far cultivated areas.

Only temperature seems to have a consistent and positive effect over current-day *Alternaria* spore concentrations in

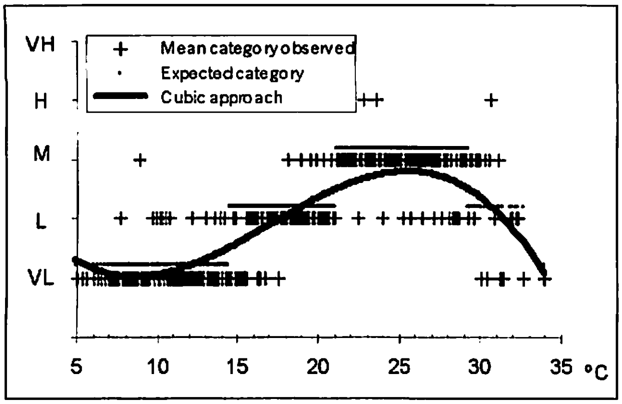

Fig. 6. Mean spore concentration levels observed for every mean temperature and curve obtained by curvefit regression analysis. Points corresponding to expected level have been moved upwards to avoid coincidence with observations.

Murcia, but evaporation and low wind velocities could have a positive effect and north-western wind a negative effect.

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