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Comparative study of airborne *Alternaria* conidia levels in two cities in Castilla-La Mancha (central Spain), and correlations with weather-related variables

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Abstract

Alternaria conidia are among the airborne biological particles known to trigger allergic respiratory diseases. The presented paper reports on a study of seasonal variations in airborne *Alternaria* conidia concentrations in 2 cities in the central Spanish region of Castilla-La Mancha, Albacete and Toledo. The influence of weather-related variables on airborne conidia levels and distribution was also analysed. Sampling was carried out from 2008-2010 using a Hirst sampler, following the methodology established by the Spanish Aerobiology Network. Annual airborne *Alternaria* conidia counts were higher in Toledo (annual mean 3,936 conidia) than in Albacete (annual mean 2,268 conidia). Conidia were detected in the air throughout the year, but levels peaked between May-September. Considerable year-on-year variations were recorded both in total annual counts and in seasonal distribution. A significant positive correlation was generally found between mean daily *Alternaria* counts and both temperature and hours of sunlight, while a significant negative correlation was recorded for relative humidity, daily and cumulative rainfall, and wind speed. Regression models indicated that between 31%-52% of the variation in airborne *Alternaria* conidia concentrations could be explained by weather-related variables.

Key words

Airborne fungal spores, *Alternaria*, seasonal variation, meteorological variables, allergy, Spain, Albacete, Toledo

INTRODUCTION

Many of the abundant and extremely diverse fungal spores present in the air give rise to diseases in plants, animals and humans, as well as spoiling food and organic materials, and contributing to the corrosion and deterioration of stone monuments and metals. These bioparticles are almost constantly present in the air, both indoors and outdoors, and are also present in domestic dust [1, 2, 3, 4]. *Alternaria* conidia are among the most commonly encountered airborne particles. *Alternaria* belongs to the class Deuteromycetes, which comprises around 50 species distributed worldwide. Many of these species are saprophytic, and are known to colonise and degrade a wide range of substrates, including paper, leather, upholstery, paint and foods which, once contaminated, may transmit mycotoxins; other species are plant pathogens that cause major economic losses in the agricultural sector by reducing crop yields and spoiling vegetables held in storage [5, 6]. A number of authors [7, 8, 9] have noted that *Alternaria* plays a major role in respiratory allergic disease, particularly in the Mediterranean area [10]. Several studies have clearly established the role of *Alternaria* conidia sensitisation in the development of symptomatic asthma which occurs primarily in children [11]. The reported prevalence of sensitisation amongst patients allergic to *Alternaria* conidia varies considerably. Studies carried out in Spain suggest that 32% of fungal-spore allergy-sufferers in Córdoba are sensitive to *Alternaria* [12], compared with 9% in Almería [13], and 18.3% in Catalonia [14]. These discrepancies in the results of epidemiological studies are attributable to significant differences in airborne spore levels and to local climate variations. It is difficult to establish precisely the minimum level of airborne *Alternaria* conidia likely to trigger symptoms in allergy-sufferers, and reported threshold levels vary widely: Eduard [15] suggests a threshold level of 10 conidia/m^3, Frankland & Davies [16] indicate a figure of 50 conidia/m^3, while, other authors [17, 18, 19] advocate a higher threshold of 100 conidia/m^3.

Since the formation and release of fungal spores is strongly influenced by local climate conditions, many authors have performed studies analysing the correlation between weather-related parameters and airborne *Alternaria* conidia concentrations in areas with different climates [20, 21, 22, 23, 24].

Given the clinical and aerobiological importance of this genus, the presented study aimed to analyse the seasonal behaviour of *Alternaria* in two cities in the central Spanish region of Castilla-La Mancha, Albacete and Toledo, by measuring airborne conidia concentrations and charting the influence of weather-related parameters.

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MATERIALS AND METHODS

Airborne *Alternaria* conidia levels were measured at 2 sampling stations in central Spain (Albacete and Toledo) between 2008-2010. The chief geographical and climatic characteristics of the sampling sites, using the classification established by Rivas-Martínez [25], are shown in Table 1. Sampling and slide analysis were carried out using the methodology developed by the Spanish Aerobiology Network [26]. *Alternaria* conidia were collected using Hirst volumetric spore traps [27]. The traps functioned continuously, sucking in air at a rate of 10 l/min, with the fungal conidia being trapped on an adhesive-coated Melinex® strip. Daily preparations were analysed under a light microscope (400x magnification), with 4 longitudinal sweeps per slide. Mean daily values were expressed as number of conidia per cubic metre of air (conidia/m^3). To obtain this, the number of pollen grains counted per unit area (N) is multiplied by a correction factor (CF) that takes into account the volume of air sampled (10 litres/minute), and the size of the microscope field of vision used (400x magnification). In this case, conida/m^3=0.5 (CF) x N, being N=number of conidia counted in 4 sweeps. *Alternaria* conidia were identified using descriptions provided in the literature [28, 29, 30].

The severity of the season was evaluated in terms of the number of days on which average spore counts exceeded the suggested threshold concentrations of: 10 conidia/m^3 [15], 50 conidia/m^3 [16] and 100 conidia/m^3 [17, 18, 19], above which spore-allergy sufferers start to show serious symptoms.

Pearson's correlation test was used to determine correlations between weather-related variables and daily conidia counts. A logarithmic transformation (log [x+1], where x = daily mean *Alternaria* conidia counts expressed as conidia/m^3) was carried out, since the data were not normally distributed. Weather data were provided by the Spanish Meteorological Agency (AEMET) and by the Department of the Environment of the Castilla-La Mancha Government. The variables selected were: mean temperature (Tmean, °C), hours of sunlight (HS, h), relative humidity (RH, %), rainfall (R, mm), accumulated rainfall over 7 days (Ac., R, mm), and wind speed (WS, m/s). One-day lag data, i.e. correlations between meteorological variables for a given day and *Alternaria* counts 1 day later, were also examined: Tmean_Lag, HS_Lag, RH_Lag, R_Lag and WS_Lag.

Following Stennett & Beggs [31] and Rodríguez-Rajo *et al.* [32], stepwise multiple regressions were performed using weather-related parameters as independent variables and daily *Alternaria* conidia counts as dependent variable, in order to determine which of these parameters, if any, accounted for the variation in airborne *Alternaria* conidia counts. The most parsimonious and least complex equation – with the best R^2 value – was selected. Regression analyses were performed at both sites for each study year, and for the study period as a whole (2008-2010).

RESULTS

Between 2008–2010, a total of 6,805 *Alternaria* conidia were identified in Albacete, and a total of 11,807 in Toledo. The behaviour of the weather-related variables most directly influencing conidia counts over the same period is shown in Figure 1. Monthly variations in mean temperature and hours of sunlight were similar in the 2 study cities: both recorded a gradual increase from May - August, peaking in July; minimum values were observed in winter (December and January). Relative humidity displayed the opposite trend, the highest values being recorded in the coldest months, and the lowest values in the warmest months. The rainfall distribution pattern was typical of the Mediterranean Region, with a period of summer drought. Annual rainfall ranged from 415 mm (Toledo, 2009) to 615 mm (Albacete, 2010). In both cities, 2010 was the wettest year and 2009 the driest. Finally, wind speed remained fairly constant over the study period, ranging between 0.7-2.3 m/s in Albacete and between 1.2-4.4 m/s in Toledo.

Toledo recorded a higher annual airborne *Alternaria* conidia count (mean 3,936) than Albacete (mean 2,268; Tab. 2). In both cities, the highest counts were found in 2008 and the lowest in 2009. Daily counts peaked between mid-May - mid-August, except in 2009 when the peak count in Albacete was not recorded until 26 September. Peak-day counts were very high: 324 conidia/m^3 in Toledo on 18/06/2008, and 263 conidia/m^3 in Albacete on 17/08/2010 (Tab. 2). Mean daily counts varied over the year in both cities, ranging between 4-12 conidia/m^3.

Table 2. Numerical description of *Alternaria* conidia concentrations in Albacete and Toledo from 2008 to 2010

		Albacete			Toledo		
		2008	2009	2010	2008	2009	2010
Total annual conidia		**2,845**	**1,596**	**2,364**	**4,527**	**3,378**	**3,902**
Daily mean concentration (conidia/m^3)		8,0	4,4	6,9	12,4	9,3	10,8
Maximum value		142	114	263	324	198	213
Day maximum value		**May 15**	**Sep 26**	**Aug 17**	**Jun 18**	**Jun 20**	**Aug 9**
No. of days	Conidia/m^3 = 0	56	94	102	47	72	119
	Conidia/m^3 ≥ 10	102	31	44	107	84	87
	Conidia/m^3 ≥ 50	5	4	8	16	15	21
	Conidia/m^3 ≥ 100	1	2	4	7	3	8

Conidia were detected in the air throughout the year and its evolution was opposite to the one followed by relative humidity (Fig. 2). *Alternaria* concentrations increased from winter to spring, while the values of the relative humidity

Table 1. Geographical, climatic and bio-geographical characteristics of study area

Station	Geographical characteristics: Altitude (m above sea level)	Geographical characteristics: Geographic location	Climatic and bio-geograhical characteristics: Annual rainfall (mm)	Mean annual temperature (ºC)	Climate	Mediterranean Region – Bio-geographical Provinces
Albacete	686	38º 58´N, 01º 51´O	336	13.6	Mediterranean continental	Mediterranean Region. Central Iberian Province (Manchean Sector)
Toledo	449	39º 51´N, 04º 02´O	382	15.4	Mediterranean continental	Mediterranean Region. Central Iberian Province (Manchean Sector) and West Iberian Province (Toledan-Taganean Sector)

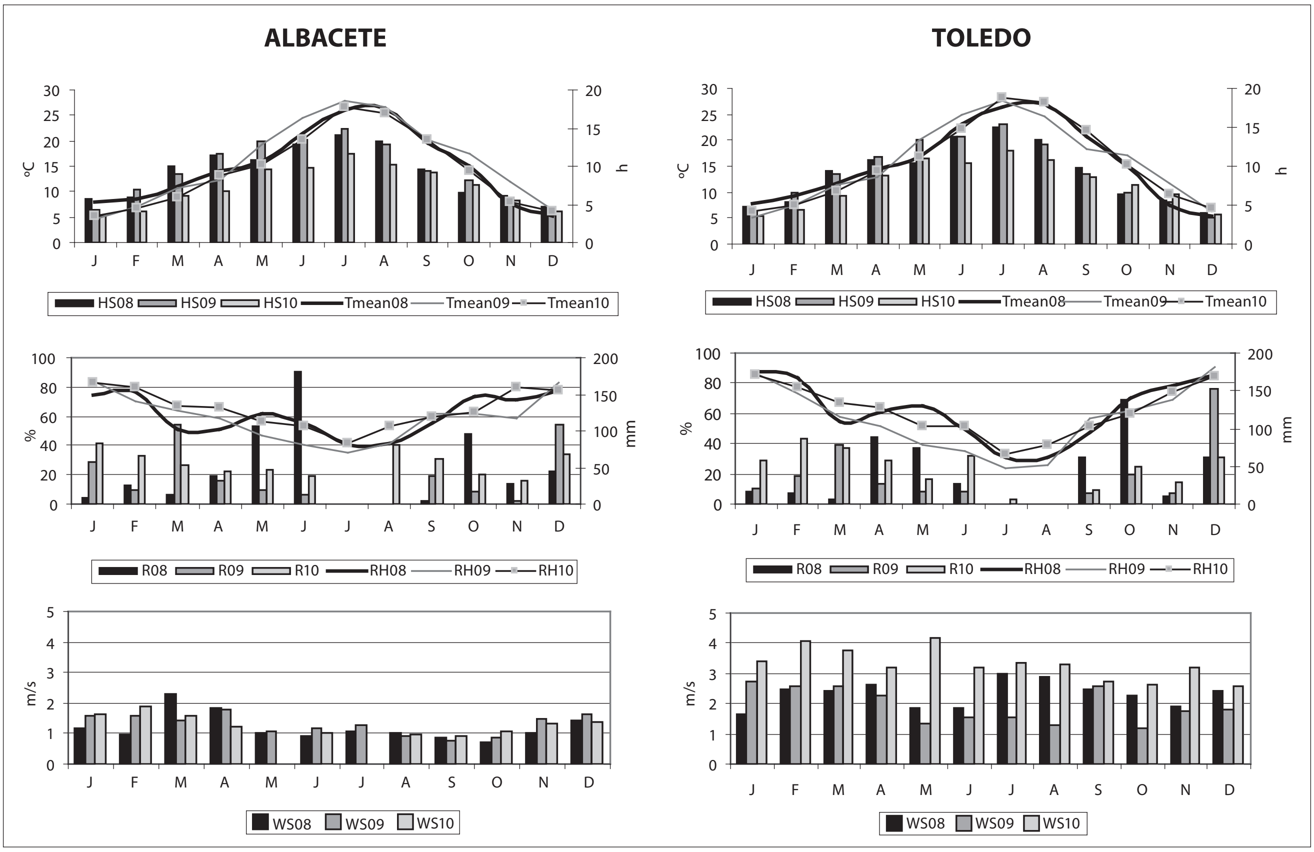

Figure 1. Mean monthly temperatures (Tmean, °C), hours of sunlight, (HS) rainfall (R, mm), relative humidity (RH, %), and wind speed (WS, m/s) from 2008-2010 in Toledo and Albacete

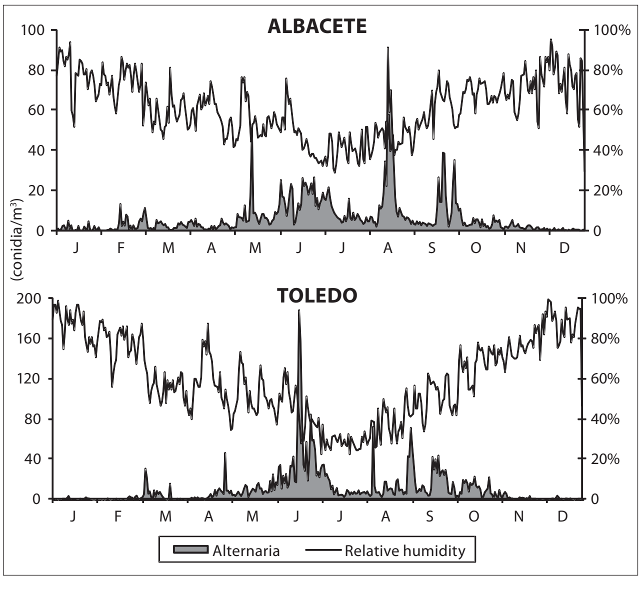

Figure 2. Mean daily values for Alternaria concentrations (conidia/m^3) and relative humidity (%) during the study period (2008-2010).

decreased. In Albacete, maximum values were recorded from May - August and from June - September in Toledo. The lowest concentrations were observed during the winter months (December-February), when monthly counts generally remained below 50 conidia/m^3.

In Albacete, the month displaying the highest counts varied from year to year: in 2008, maximum monthly counts were recorded in June, closely followed by July and May; in 2009, monthly maxima were observed in September, followed by November; and in 2010, August followed by June. In Toledo, by contrast, maximum monthly counts were recorded either in June (2008) or in June and September, with very similar values (2009 and 2010; Fig. 3).

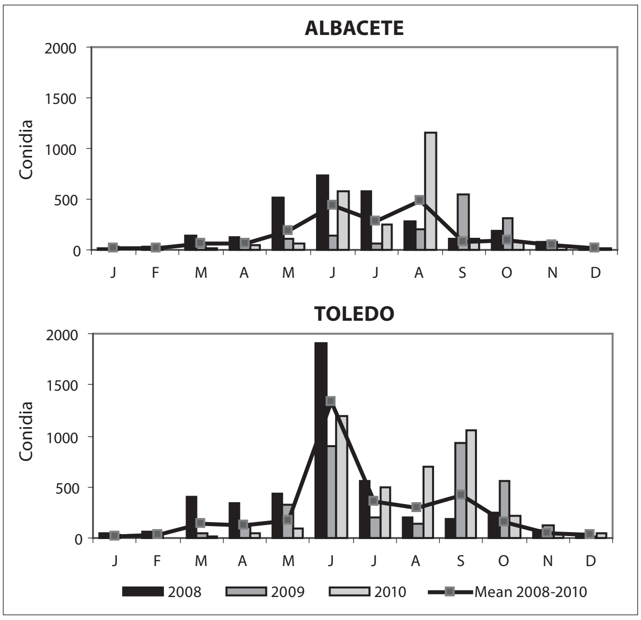

Figure 3. Monthly (January–December) total *Alternaria* spore counts and monthly mean *Alternaria* spore counts from 2008-2010 in Albacete and Toledo

Table 3. Pearson´s correlation coefficients for daily *Alternaria* conidia counts and weather-related variables. Weather-related variables on a given day and *Alternaria* counts 1 day later were also examined (_Lag). ** (p<0.01), * (p<0.05)

	Albacete				Toledo			
	2008	2009	2010	Mean	2008	2009	2010	Mean
Tmean	**0.548****	**0.569****	**0.682****	**0.581****	**0.519****	**0.579****	**0.702****	**0.595****
Tmean_Lag	**0.524****	**0.555****	**0.672****	**0.565****	**0.515****	**0.565****	**0.693****	**0.587****
HS	**0.501****	**0.440****	**0.534****	**0.487****	**0.508****	**0.263****	**0.476****	**0.418****
HS_Lag	**0.488****	**0.417****	**0.520****	**0.471****	**0.512****	**0.239****	**0.437****	**0.396****
RH	**-0.416****	**-0.513****	**-0.513****	**-0.469****	**-0.431****	**-0.552****	**-0.541****	**-0.492****
RH_Lag	**-0.366****	**-0.434****	**-0.477****	**-0.415****	**-0.384****	**-0.485****	**-0.496****	**-0.441****
R	0.069	**-0.246****	-0.082	-0.039	-0.022	**-0.235****	**-0.207****	**-0.147****
Ac. R	**0.282***	**-0.275****	-0.073	**-0.141***	0.007	**-0.231****	**-0.254****	**-0.167****
R_Lag	0.055	**-0.186****	**-0.116***	-0.042	-0.052	**-0.191****	**-0.215****	**-0.147****
WS	-0.035	**-0.211****	**-0.247****	**-0.169****	**0.111***	**-0.184****	-0.070	**-0.087****
WS_Lag	-0.069	**-0.258****	**-0.281****	**-0.205****	-0.040	**-0.234****	**-0.144****	**-0.163****

The number of days on which counts exceeded the threshold above which spore-allergy sufferers start to show serious symptoms was higher in Toledo than in Albacete (Table 2). In Albacete, daily mean counts exceeding 50 conidia/m^3 were recorded on only 10 days per year over the 3-year study period, compared with between 16 - 21 days per year in Toledo. Evidently, when the threshold concentration was set at 10 conidia/m^3, the number of days recording above-threshold counts increased significantly.

Correlations between mean daily *Alternaria* spore counts and meteorological parameters are shown in Table 3. In both cities and in all study years, a highly-significant positive correlation was observed between heat-related variables (mean temperature and hours of sunlight) and spore counts both on the same day and one day later. By contrast, a significant negative correlation was recorded between spore counts and relative humidity, rainfall, cumulative rainfall and wind speed, although correlations with rainfall and wind speed were less consistent. Exceptionally, a significant positive correlation was observed with cumulated rainfall in Albacete (2008) and with wind speed in Toledo (2008).

The regression results shown in Table 4 suggest that variation in *Alternaria* conidia concentrations can be accounted for to a large extent by meteorological variables (31-52%). All the resulting prediction equations included mean temperature in the first step; in the second step, the predictive variable differed as a function of study year and sampling site. In the regression equations proposed for Albacete, the first independent variable (Tmean), accounted for 31% of the variation in airborne *Alternaria* conidia counts. In Toledo, the equations selected for the study years also included as variables both Tmean and WS_Lag (R^2= 0.37%).

Table 4. Regression models for *Alternaria* conidia in Albacete and Toledo (2008-2010)

		Predictive equations	R^2
Albacete	2008	$y = 0.036\ Tmean + 0.048\ HS - 0.500$	0.32
	2009	$y = 0.047\ Tmean - 0.117\ WS\_Lag - 0.473$	0.33
	2010	$y = 0.069\ Tmean + 0.047\ HS\_Lag - 1.215$	0.50
	2008-2010	$y = 0.057\ Tmean - 0.718$	0.31
Toledo	2008	$y = 0.145\ HS\_Lag + 0.009\ RH\_Lag - 1.330$	0.31
	2009	$y = 0.034\ Tmean - 0.010\ RH + 0.332$	0.37
	2010	$y = 0.081\ Tmean - 0.029\ R\_Lag - 1.070$	0.52
	2008-2010	$y = 0.063\ Tmean - 0.080\ WS\_Lag - 0.432$	0.37

DISCUSSION

Airborne conidia counts were higher in Toledo than in Albacete, perhaps due to the greater local abundance and availability of host plants, to the proximity of the River Tagus, which affords an environment with abundant hygro-nitrophyllous herbaceous communities, suitable for the development of *Alternaria* conidia, and to the location of the spore trap at the edge of the city, close to the riverbank and to cropland. As a number of authors have noted [33], low levels of urbanisation favour a greater abundance of airborne conidia.

In Castilla-La Mancha, the highest *Alternaria* conidia concentrations were detected from May - September, coinciding with optimal temperature (15-25°C) and humidity (30-60%) conditions for the development of this fungus. The lowest concentrations were recorded in winter, when low temperatures inhibit spore formation. Peak counts were observed from May - August in Albacete and from June to September in Toledo; similar findings have been reported for other Spanish cities, including Almería, León, or Palencia [13, 34, 35]. By contrast, in southern inland cities such as Córdoba and Granada, a marked drop in conidia counts was recorded in July and August, due to very high temperatures [20, 24]. Elsewhere in Europe, maximum airborne *Alternaria* conidia counts in Sweden [23] and Poland [22] are observed in July and August. Seasonal variations in conidia counts may be attributed, like quantitative variations, to differences in local climate which strongly influence the distribution of airborne conidia, and to the presence of habitats conducive to the growth of *Alternaria*, a common pathogen of cereals, oil crops, fruits, vegetables, and ornamentals [36].

In both cities, marked year-on-year differences were observed in monthly spore counts. This may be due in part to differences in weather conditions: in Albacete, for example, higher May - July counts in 2008 compared to the following 2 years may reflect greater rainfall in spring, while the high monthly count recorded in August 2010 coincided with an increase in rainfall unusual for August in the Mediterranean region (81.5 mm). Monthly counts for September and October were higher in 2009, perhaps reflecting an increase in mean temperatures compared to 2008 and 2010. In Toledo, higher counts were recorded in June - August 2010 and March-April 2008 compared to the same periods in other years; this may be due to an increase in mean temperatures, which would favour conidia release.

Airborne *Alternaria* conidia counts in Albacete and Toledo, cities situated in the central region of Castilla-La Mancha, were lower than those recorded in northern and southern Spain, where annual totals range between 4,000 and 33,000 conidia [2]. In other European areas (e.g. in Poland), annual totals are far higher (14,000-28,000 conidia depending on the year) [22], and in Ankara (Turkey) the total count in 1990 was 59,735 [37]. These quantitative differences between geographical areas may be due both to differences in local climate and to the proximity of the spore trap to damp areas containing organic material, such as crops, gardens, orchards or decaying plants, the preferred habitat of *Alternaria* fungi [38, 39].

With regard to allergy risk, it should be noted that although *Alternaria* conidia account for only a small proportion of total airborne particles in central Spain, they can trigger symptoms in a large number of allergy-sufferers. The risk of developing allergic symptoms is greatest from May - September, both in Albacete and Toledo; in these months, threshold concentrations were exceeded on a number of days. Lower maximum daily counts are reported for other Spanish cities, including Santiago de Compostela and León [40, 41], where daily counts never exceed 25 conidia/m^3. In Trieste (north-east Italy), by contrast, daily counts of over 100 conidia/ m^3 are recorded on an average of 13 days [42], i.e. more frequently than in either Albacete or Toledo.

Analysis of the correlations between mean daily *Alternaria* spore counts and weather-related parameters disclosed that an increase in temperature and hours of sunlight had a positive influence on counts. Similar findings have been reported in studies both in Spain [24, 43, 44] and elsewhere [22, 45]. By contrast, relative humidity and both daily and cumulative rainfall had a negative impact on daily spore counts. A number of studies have highlighted the adverse effect of these parameters on airborne *Alternaria* conidia levels [20, 32, 46]. The results obtained in the presented study suggest that although daily and weekly cumulative rainfall are associated with a fall in airborne spore counts, earlier heavy rainfall over a long period favours the subsequent presence of airborne conidia. The significant negative correlation with wind speed, indicating that low speeds are associated with higher counts, has also been reported by other authors [31].

In all years – except 2008 in Toledo – the variable Tmean accounted for most of the variation in airborne *Alternaria* conidia counts: in both Albacete and Toledo, the regression model constructed for the study period as a whole explained 31% and 37%, respectively, of the variation in counts. Higher percentages were recorded in some years, reaching 50% in Albacete in 2010 and 52% in Toledo, also in 2010. Similar R^2 values are reported by other authors, e.g. Stennett & Beggs [31] in Sydney, Australia, found that 31.3% of the variation was explained in a regression model using 4 independent variables (mean temperature, rainfall, and minimum and maximum relative humidity), while Burch & Levetin [47] in Tulsa, USA, noted that 36.6 % of the variation in airborne conidia levels was explained by 3 variables (temperature, dew point temperature, and air pressure).

The presented study concerns results related to 3 years; however, further research over a longer period is required in order to establish behaviour patterns and construct more accurate prediction models.

CONCLUSIONS

In the 2 central Spanish cities studied, Albacete and Toledo, the highest airborne *Alternaria* conidia counts were recorded in late spring and summer. Total counts during the period 2008-2010 were considerably higher in Toledo than in Albacete, probably due to the presence of crops and other vegetation in the vicinity of the spore trap on the outskirts of the city.

Weather-related parameters clearly influenced airborne conidia counts and their distribution over the year. The prediction equations presented here accounted for between 31% - 52% of the variation in counts. These findings are of interest to allergologists and patients sensitive to these conidia, in that they provide an indication of the periods when the highest *Alternaria* counts may be expected.

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