

Airborne fungal spores of *Alternaria*, meteorological parameters and predicting variables

Farah Filali Ben Sidel · Hassan Bouziane · Maria del Mar Trigo ·
Fatima El Haskouri · Fadoua Bardei · Abdelbari Redouane ·
Mohamed Kadiri · Hassane Riadi · Mohamed Kazzaz

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Abstract *Alternaria* is frequently found as airborne fungal spores and is recognized as an important cause of respiratory allergies. The aerobiological monitoring of fungal spores was performed using a Burkard volumetric spore traps. To establish predicting variables for daily and weakly spore counts, a stepwise multiple regression between spore concentrations and independent variables (meteorological parameters and lagged values from the series of spore concentrations: previous day or week concentration (Alt_{t-1}) and mean concentration of the same day or week in other years (C_{mean})) was made with data obtained during 2009–2011. *Alternaria* conidia are present throughout the year in the atmosphere of Tetouan, although they show important seasonal fluctuations. The highest levels of *Alternaria* spores were recorded during the spring and summer or autumn. *Alternaria* showed maximum daily values in April, May or October depending on year. When the spore variables of *Alternaria*, namely C_{mean} and Alt_{t-1} , and meteorological parameters were included in the equation, the resulting R^2 satisfactorily predict future concentrations for 55.5 to 81.6 % during the main spore season and the pre-peak 2. In the predictive model using weekly values, the adjusted R^2 varied from 0.655 to 0.676. The Wilcoxon test

was used to compare the results from the expected values and the pre-peak spore data or weekly values for 2012, indicating that there were no significant differences between series compared. This test showed the C_{mean} , Alt_{t-1} , frequency of the wind third quadrant, maximum wind speed and minimum relative humidity as the most efficient independent variables to forecast the overall trend of this spore in the air.

Keywords Fungal spores · *Alternaria* · Aerobiology · Meteorological parameters · Modelling · Tetouan · Morocco

Introduction

Alternaria, an anamorph of ascomycetes that produce dry conidia, is a common allergenic mould (Kurup et al. 2000; Bisht et al. 2003). It contains numerous plant pathogens which are mostly host-specific causing economic losses on a large variety of crops (Domsch et al. 1993, Infante et al. 1999; Logrieco et al. 2003; Thomma 2003). Exposure to high numbers of *Alternaria alternata* spores can trigger severe asthma exacerbations and is a risk factor for respiratory diseases in *A. alternata*-sensitized asthmatic children and young adults (O'Hollaren et al. 1991). The application of aerobiological surveys of airborne fungi to agricultural and allergy studies has proved importance, as the determination of mould counts is of great importance and concerns because of their adverse crops and health impacts (D'Amato et al. 1997; Kurup et al. 2002).

The outdoor air spora of a particular region depends on environmental factors such as temperature, moisture, nutrient availability, plant materials, circadian periodicity and seasons. Most of the statistical analysis of spore data is based on the Spearman correlation test due to the nonnormality of the spore distribution (Sabariego et al. 2000; Das and Gupta-Bhattacharya 2008; Rodríguez-Rajo et al. 2005; Sánchez-

F. Filali Ben Sidel · H. Bouziane (✉) · F. El Haskouri · F. Bardei ·
M. Kadiri · H. Riadi · M. Kazzaz
Laboratory of Diversity and Conservation of Biological Systems,
Faculty of Sciences, Mhannech II, University Abdelmalek Essaâdi,
Postal Code 2121 Tetouan, Morocco
e-mail: hasbouz@hotmail.com

M. del Mar Trigo
Department of Plant Biology, University of Malaga, P.O. Box 59,
29080 Malaga, Spain

A. Redouane
Laboratory of Energetic, Physics Department, Faculty of Sciences,
Mhannech II, University Abdelmalek Essaâdi, Postal Code
2121 Tetouan, Morocco

Reyes et al. 2009; Oliveira et al. 2010). Nevertheless, linear and stepwise multiple regressions have also been underway (Grinn-Gofroń and Rapijko 2009; Recio et al. 2012). Most of these studies are working on these models which almost uniformly use meteorological data as the main controlling independent variables.

According to Damialis and Gioulekas (2006), forecasting models of airborne fungal spore release and dispersion are few and most of them display low predictability. The importance of predictive models for both short- and long-term variation in spore concentrations lies in the ability to prevent exposure and symptoms. They are also useful to make appropriate preventive medical treatments. In this study, an attention was paid to the spore concentration mean value for that day or week as do Recio et al. (2002) and to lagged values from the series of daily or weekly spore concentrations.

The aim of this study was to determine the annual and seasonal variation of atmospheric *Alternaria* spores in Tetouan (Morocco) during 2009–2012, to examine the relationship between spore levels and meteorological factors and to create forecasting models to obtain the most accurate predictions of the daily and weekly spore concentrations of *Alternaria*.

Material and methods

The province of Tetouan is located in the eastern sector of the Tingitan Peninsula in the northwest of Morocco (Fig. 1), covering an area of 2,574 km². In bioclimatic terms, it belongs to the thermomediterranean climate. It is characterized by two seasons, the first humid and fresh from October to April and the second drier and hot from May to September. For the years

2009–2012, the average mean temperature was 19.2 °C and mean annual rainfall was 765.5 mm. The mean annual maximum temperature varied between 22.3 and 23.1 °C, whereas in the case of the minimum temperature, the averages ranged from 14.3 to 17.6 °C. The coldest months are January, February or December, respectively. The warmest month corresponds to July or August. The city, far 10 km from the Mediterranean Sea, is located between two mountain ranges; therefore, the prevailing winds are those blowing from the NE (known locally as “Chergui”) and SW (locally known as “Gharbi”) (Aboulaich et al. 2013).

Aerobiological sampling was performed during 2009–2012 using Burkard 7-day volumetric spore traps located on the flat roof of the Department of Biology of the Faculty of Sciences of Tetouan 15 m above ground level. The sampler was calibrated to aspirate an air flow of 10 l/min. To collect daily airborne spores, a trapping surface of Melinex film coated with 2 % silicon solution was mounted on the drum rotating inside the sampler at a speed of 2 mm/h. The drum was changed weekly and the exposed tape was cut into seven segments for daily spore counts. The 24-h slide was analysed under a microscope with a ×40 objective every 2 h using two longitudinal traverse methods according to the Spanish Aero-biological Network (REA). Daily mean concentrations were expressed as the number of spores per cubic metre of air. The main spore season (MSS) was determined by the 90 % method of Nilsson and Person (1981) taking the beginning and end of the MSS as 5 and 95 % of the annual accumulated sums.

The correlation coefficient between mean daily spore concentrations and meteorological parameter values was calculated using Spearman's correlation test for statistical significance $p < 0.01$ and $p < 0.05$. Values were computed by the program Statistica (Softwear program). The meteorological parameters

Fig. 1 Location of the sampling site, in Tetouan, Morocco



Table 1 Monthly total of *Alternaria* spores in the air of Tetouan, 2009–2012

Month	Monthly total			
	2009	2010	2011	2012
January	299.16	194.4	645.84	444.96
February	170.64	233.28	415.8	380.16
March	564.84	451.44	740.88	741.96
April	821.88	3,495.96	1,626.48	2,262.60
May	1,455.84	3,353.4	4,742.28	2,849.04
June	1,902.24	5,651.64	3,786.48	1,684.44
July	1,531.44	3,222.72	1,897.56	1,534.68
August	949.32	4,330.8	3,471.12	2,223.72
September	1,959.12	2,184.84	2,689.2	2,504.79
October	2,969.3	2,757.24	1,926.72	4,288.68
November	1,181.52	959.04	1,323	896.40
December	506.52	846.72	784.08	664.20
Total	14,312	27,681	24,049	20,476
Mean±SD	1,193±816.65	2,307±1,783.85	2,004±1,393.87	1,706±1,177.08

used were maximum temperature ($T_{\max}$), mean temperature (T_{mean}), minimum temperature ($T_{\min}$), maximum relative humidity ($RH_{\max}$), mean relative humidity (RH_{mean}), minimum relative humidity ($RH_{\min}$), precipitation, mean and maximum wind speed (WS_{mean} and $WS_{\max}$), frequencies of four directions of wind (NE, SE, NW, SW) and calm. Meteorological data were obtained from the Physics Department of the Faculty of Science, which has an automatic meteorological station located 500 m from the sampling site.

A multiple stepwise regression by backward elimination was made to evaluate the parameters forming part of the mathematical model that enables us to predict *Alternaria* spore concentrations using data from 2012 as external values to validate the forecast model. This analysis was applied between dependent variable (daily *Alternaria* spore concentrations of the MSS, weekly means of the year and averaged daily values of the second pre-peak period) and 15 independent variables. The pre-peak 2 was defined as the second period when the daily spore concentration curve starts to increase accumulating 100 sp/m³ until a maximal value is reached. The pre-peak 1 was rejected as no good results were obtained when it was used for regression analysis. The 15

independent variables were firstly the lagged values from the series of spore concentrations, previous day or week concentration (Alt_{t-1}) and mean concentration for the same day or week of other years 2009–2011 (C_{mean}) and secondly the same meteorological parameters selected above for the correlation analysis at the exception of T_{mean} . The Wilcoxon test was made to select the reasonable predictor variables by comparing averages of observed and predicted series. Spearman's correlation test was also performed to estimate the degree of association between these two series.

Results

Spores data

Spores of *Alternaria* were found in the air of Tetouan throughout the entire year with low values during winter. The highest number of spores was recorded in 2010 with 27,681 spores, whereas the lowest value occurred in 2009 with 14,312 spores (Table 1). The monthly spore levels showed the highest spore concentrations during spring

Table 2 Data of the main spore season (MSS) of *Alternaria*, 2009–2012

	Main spore season (MSS)				Spore peak	
	MSS-start	MSS-end	Duration day	Total recorded	Day	Value
2009	17 March	27 November	256	13,060	08 October	384
2010	28 March	09 December	257	26,380	26 April	767
2011	15 March	14 November	245	21,645	19 April	471
2012	06 March	16 November	256	18,517	10 October	399

MSS-start starting date of MSS,
MSS-end end date of MSS

and summer or autumn with two monthly maximum concentrations. The first maximal load is observed in May for 2011 and 2012 or June for 2009 and 2010 and the second in August for 2010 and 2011 or October for 2009 and 2012 (Table 1).

During the four analysed years, the MSS lasted between 245 and 257 days. Start dates of the spore season varied from the beginning to the end of March. End dates were in the second fortnight of November in 2009, 2011 and 2011 and in 9 December in 2010 (Table 2). The maximum daily spore concentration reached 384 sp/m³ on 8 October in 2009, 399 sp/m³ on 10 October in 2012, 471 sp/m³ on 19 May in 2011 and 767 sp/m³ on 26 April in 2010 (Table 2 and Fig. 2).

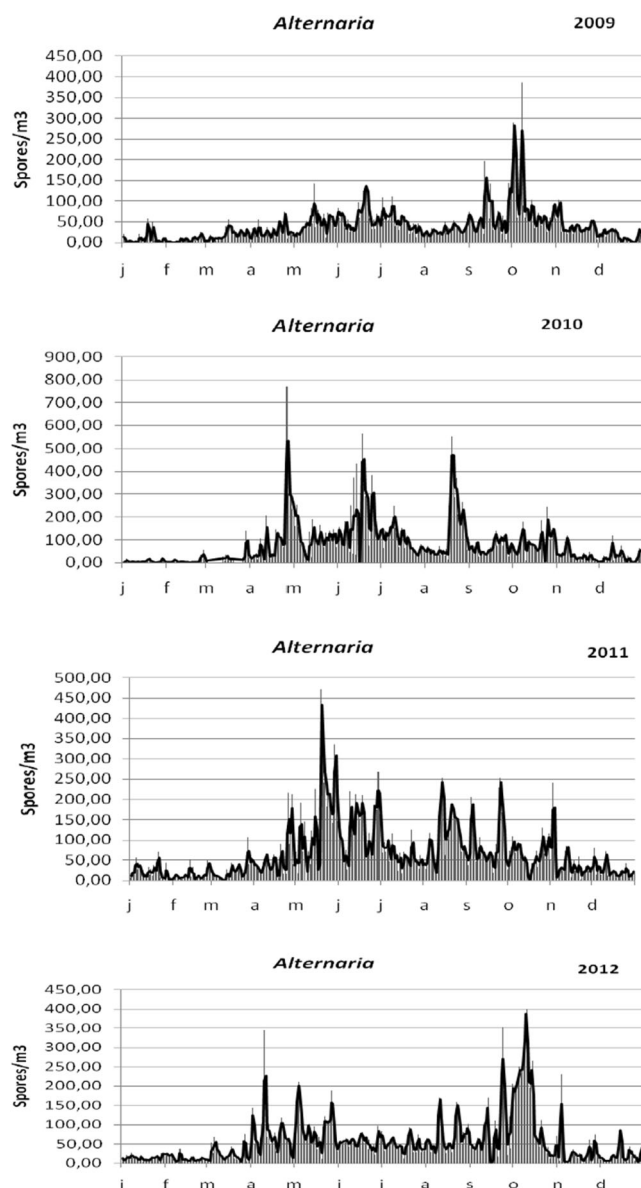


Fig. 2 Daily mean values of *Alternaria* spores, 2009–2012

Correlation with meteorological parameters

Spore concentration of *Alternaria* showed a positive and highly significant correlation with $T_{\min}$, T_{mean} and $T_{\max}$ with a significance level of $p < 0.01$ (Table 3). A negative and statically significant to highly significant correlation was observed between daily spore counts of *Alternaria* and minimum and mean relative humidity, the correlation coefficients being lower than for temperature. Only in one year (2010), it was observed that the maximum relative humidity correlated negatively with a highly significant coefficient. In all years analysed, *Alternaria* presented a negative and significant to highly significant correlation with rainfall. The association between wind speed and *Alternaria* spore concentrations was always positive and significant to highly significant. A highly significant correlation was found between the NW (fourth quadrant) wind direction and spore levels of *Alternaria* in 2012 and all the period in 2009–2012, whereas the negative influence of the NE (first quadrant) and SE (second quadrant) wind direction was observed in 2012 and 2009, respectively.

Stepwise multiple regression

In the stepwise backward elimination regression, which not included T_{mean} , the percentage variance explained up to 55.1 % for the MSS, 81.6 % for the pre-peak and 67.6 % for the weekly values (Table 4). When more meteorological parameters were eliminated from the equation, the same regression equations and coefficients were obtained for models 2 to 4 for the MSS. However, the resulting R^2 satisfactory predicts future concentration with an adjusted regression coefficient ranging from 53.2 to 66.9 % when averaged daily data for the pre-peak 2 and averaged weekly data were used (Table 4). The four models obtained for daily values of the MSS were rejected since statistically significant differences were found between the predicted and observed fungal spore counts (Table 5).

With regard to the daily values of the pre-peak and weekly values of the year, the Wilcoxon test was used to compare the results from the expected values and observed data for 2012, indicating that there were no significant differences between series compared when models 2 and 3 were selected, respectively (Table 5). In the case of the pre-peak, the meteorological parameters that the stepwise regression analysis selected were Fr 3Q and $WS_{\max}$ (Table 5). In the stepwise backward elimination regression modelling approach performed with weekly values, the variable $RH_{\min}$ was maintained in the third model and R^2 reached 66.9 % (Table 5). The Spearman's correlation test indicated a very high degree of association (0.913)

Table 3 Spearman's correlation coefficients between *Alternaria* spore concentrations and meteorological factors

	2009	2010	2011	2012	2009–2012
Minimum temperature	0.42**	0.48**	0.51**	0.29**	0.31**
Mean temperature	0.43**	0.52**	0.42**	0.41**	0.39**
Maximum temperature	0.45**	0.50**	0.51**	0.40**	0.41**
Minimum relative humidity	-0.16**	-0.16**	-0.13*	-0.16**	-0.10*
Mean relative humidity	-0.12*	-0.18**	-0.14*	-0.14**	-0.10*
Maximum relative humidity	-0.08	-0.14**	-0.06	-0.05	-0.01
Maximum wind speed	0.18**	0.16**	0.18**	0.17*	0.12*
Mean wind speed	0.18*	0.17**	0.20**	0.19**	0.14*
Rainfall	-0.13*	-0.21**	-0.15**	-0.15**	-0.10*
% NE	0.03	0.07	0.04	-0.16**	0.02
% SE	-0.18**	-0.07	-0.07	-0.02	-0.06
% SW	0.07	-0.02	-0.03	0.07	-0.01
% NW	-0.07	0.03	0.07	0.32**	0.13**
Calm	-0.02	-0.04	-0.05	0.01	-0.02

* $p < 0.05$; ** $p < 0.01$

between the observed and expected series showing model 3 ($Alternaria = 0.333 Alt_{t-1} + 0.784 C_{mean} + 5.018 RH_{min} - 305.317$) as the most efficient to predict the overall trend of *Alternaria* spores in the air (Table 5). Figure 3 shows the predicted spore season for 2012. Compared to the observation, the pattern fits quite well, but the concentrations are overestimated except for the beginning.

Discussion

Fungal spores of *Alternaria* are ubiquitous components of both indoor and outdoor air samples; they are considered to be important causes of human allergic diseases and plants' pathogenic infections (D'Amato et al. 1997; Kurup et al. 2002; Ragab and Clement 2007).

Table 4 Multiple regression models proposed for predicting daily *Alternaria* spore concentration for the MSS, the pre-peak and the weekly concentrations

Models	Equations	R^2
Main spore season		
1 ^a	$Alternaria = 0.461 Alt_{t-1} + 0.659 C_{mean} - 9.622$	0.551
2–4 ^a	$Alternaria = 0.461 Alt_{t-1} + 0.659 C_{mean} - 9.622$	0.551
Pre-peak		
1 ^b	$Alternaria = -0.155 RH_{min} + 0.630 T_{min} - 0.070 WS_{max} + 0.020 Fr 2Q + 0.045 Fr 4Q + 0.021 Calme + 22.935$	0.816
2–3 ^b	$Alternaria = 0.554 Alt_{t-1} + 0.449 C_{mean} + 0.228 Fr 3Q - 0.605 WS_{max} - 1.542$	0.532
Weekly concentrations		
1 ^c	$Alternaria = 0.328 Alt_{t-1} + 0.792 C_{mean} + 6.581 RH_{mean} - 489.272$	0.676
2 ^c	$Alternaria = 0.324 Alt_{t-1} + 0.756 C_{mean} - 33.496$	0.655
3 ^c	$Alternaria = 0.333 Alt_{t-1} + 0.784 C_{mean} + 5.018 RH_{min} - 305.317$	0.669

R^2 regression coefficient, Alt_{t-1} *Alternaria* spore concentration recorded 1 day before, C_{mean} mean concentration for the same day, T_{min} minimum temperature, WS_{max} maximum wind speed, $Fr 2Q$ frequency of the wind second quadrant (SE), $Fr 3Q$ frequency of the wind third quadrant (SW), RH_{max} maximum relative humidity; frequency of the wind fourth quadrant (NW), RH_{mean} mean relative humidity, RH_{min} minimum relative humidity

a) Parameters eliminated for Main Spore Season

1: T_{mean} ; 2: T_{mean} and T_{min} ; 3: T_{mean} , T_{min} , RH_{mean} ; 4: T_{mean} , T_{min} , RH_{mean} and RH_{max}

b) Parameters eliminated for pre-peak

1: T_{mean} ; 2: T_{mean} and T_{min} ; 3: T_{mean} , T_{min} , RH_{mean} and RH_{max}

c) Parameters eliminated for weekly concentrations

1: T_{mean} and T_{min} ; 2: T_{mean} , T_{min} and H_{mean} ; 3: T_{mean} , T_{min} , H_{mean} and H_{max}

Table 5 Spearman's correlation coefficients and comparison of averages by the Wilcoxon test between the main spore season, the pre-peak period and *Alternaria* spore weekly values of the year 2012 (observed) and data from the linear regression equations (predicted)

Spearman's correlation (<i>r</i>)		Wilcoxon test			
		Rank+	Rank−	z	p
Main Spore Season					
Observed 2012 vs predicted Mod. 1 ^a	0.686***	149	107	−2.587	0.010
Observed 2012 vs predicted Mod. 2–4 ^a	0.686***	149	107	−2.587	0.010
Pre-peak					
Observed 2012 vs predicted Mod. 1 ^b	0.020	08	61	−6.646	0.000 s
Observed 2012 vs predicted Mod. 2–3 ^b	0.703***	36	33	−0.141	0.888 n.s
Weekly concentrations					
Observed 2012 vs predicted Mod. 1 ^c	0.776***	25	27	−0.310	0.757 n.s
Observed 2012 vs predicted Mod. 2 ^c	0.805***	24	28	−0.437	0.662 n.s
Observed 2012 vs predicted Mod. 3 ^c	0.788***	24	28	−0.109	0.913 n.s

n.s. not significant

^a Parameters eliminated for main spore season: (1) T_{mean} ; (2) T_{mean} and T_{min} ; (3) T_{mean} , T_{min} , RH_{mean} ; (4) T_{mean} , T_{min} , RH_{mean} and RH_{max}

^b Parameters eliminated for pre-peak: (1) T_{mean} ; (2) T_{mean} and T_{min} ; (3) T_{mean} , T_{min} , RH_{mean} and RH_{max}

^c Parameters eliminated for weekly concentrations: (1) T_{mean} and T_{min} ; (2) T_{mean} , T_{min} and H_{mean} ; (3): T_{mean} , T_{min} , H_{mean} and H_{max}

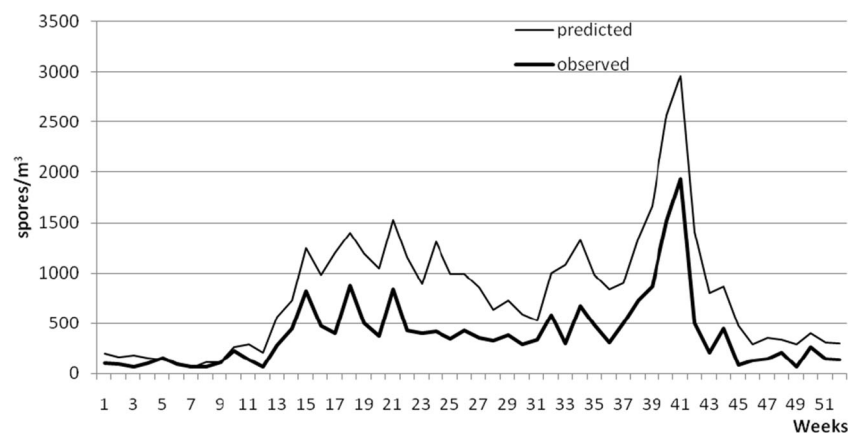
*** $p < 0.001$

Alternaria spores are the fourth most frequent spores in the air of Tetouan representing around 4 % of the annual total (Bardei et al. 2013). The spore season shows that the annual spore index varies between 14,312 and 27,681. This annual amount is generally comparable to the levels that are observed in the Catalan locations of Spain as Bellaterra, Girona and Manresa, where the annual spore index ranged between 12,511 and 21,770 (De Linares et al. 2010). The total annual value recorded for *Alternaria* spore in Tetouan is higher than the levels that are recorded in the NW Iberian Peninsula (Rodríguez-Rajo et al. 2005; Aira et al. 2008) or in Portugal (Oliveira et al. 2010). However, a spore index of 50,000 was observed by Maya-Manzano et al. (2012) in Merida (Spain). Levels of *Alternaria* registered in the sampling stations with a

rural environment were greater than those of urban stations (Oliveira et al. 2009). In this work, sampling station is located nearby a semi-rural area, thus explain the higher loads observed in Tetouan.

Atmospheric *Alternaria* spore concentrations varied according to the season with highest levels in spring and summer or autumn. This maximal load pattern is found in the Mediterranean region as observed in this study (Cosentino et al. 1995; Angulo-Romero et al. 1999; Giner et al. 2001; Maya-Manzano et al. 2012). In European countries such as Poland, Sweden, England and Spain, seasonal variation is found with only one defined period (Grinn-Gofroń and Rapiejko 2009; Skjøth et al. 2012; Corden et al. 2003; Rodríguez-Rajo et al. 2005; Aira et al. 2008; De Linares et al. 2010). The maximal

Fig. 3 Graphic representation of *Alternaria* weekly concentrations of 2012 (observed) and predicted values developed by the third model



seasonal spore counts could be related to spring blooms, plant withering due to summer drought and autumn decomposition of the vegetation. An increase in spore counts is also associated with mown grass and wheat harvesting (Friesen et al. 2001; Corden et al. 2003). Mitakakis et al. (2001) have also observed *Alternaria* burst during mowing and harvesting of grass in Australia. However, averaged daily spore concentrations of *Alternaria* showed many peaks due to numerous species of the genera, their different locations and to specific meteorological conditions.

Among meteorological parameters that influence *Alternaria* spore fluctuations, air temperature is the most important as the highest Spearman's coefficients were obtained with this factor. Similar results found by other authors in other countries were previously reported (Rodríguez-Rajo et al. 2005; Aira et al. 2008; Grinn-Gofroń and Rapiejko 2009; Sánchez-Reyes et al. 2009; Oliveira et al. 2010; Recio et al. 2012). Negative correlations were obtained between *Alternaria* levels and relative humidity and rainfall. This means that spores are released from the conidiophores bearing conidia under conditions of high temperature and low relative humidity. An increase in the relative humidity and precipitations is related to a decrease in the spore content of *Alternaria* in the atmosphere. However, relative humidity and rainfall occurring in previous days are thought to favour *Alternaria* growth and sporulation (Aira et al. 2008; De Linares et al. 2010; Recio et al. 2012). Our results showed significant correlations between *Alternaria* spores and wind, positive for speed and NW and negative with NE and SE. Lower *Alternaria* counts were registered when the wind has been blowing from the Mediterranean Sea (NE). Concentrations were found to be lower at the coastal sites by many authors (Corden et al. 2003; Aira et al. 2008; Oliveira et al. 2010). *Alternaria* conidia are "dry air spores" and high concentrations generally occurred when temperature and wind speed were enough to break conidial chains (Jones and Harrison 2004).

The forecast model proposed for the daily (namely for the pre-peak) and weekly evolution of *Alternaria* spores in the city of Tetouan indicates that both the mean spore concentration for the same day in other years and spore concentration on the previous day or week, as well as frequency of the wind third quadrant, maximum wind speed and minimum relative humidity, are the most appropriate variables for predicting the fluctuation of *Alternaria* spores in the air. The highest significant correlation between the two numerical series (observed and expected) confirmed that both presented similar behaviour patterns. The Wilcoxon test proved that there were no significant differences when models 2 and 3 were selected, verifying that the regression models proposed were valid for predicting *Alternaria* spore in the atmosphere of Tetouan. This was mainly reflected by the third model, which obtained the best results, with a high percentage of forecast (66.9 %) validated with weekly data from 2012 (Table 5).

Autoregressive models were processed by Damialis and Gioulekas (2006). According to these authors, *Alternaria* seems to have a direct relationship with minimum temperature. This meteorological parameter was selected only in one model that showed significant differences between observed and predicted values in this study. Based on the logit regression model, it was observed by De Linares et al. (2010) that the rain occurring in the three previous days enhances the increase of airborne spores of *Alternaria*. However, for these authors, the rain in the same days provokes a decrease in the number of air particles. This result was corroborated by our correlation analysis. Aira et al. (2008) carried out a multiple regression analysis and the percentage variance explained 34.5 % for *Alternaria* spores. The results obtained indicate significance differences between the spore values observed and the data predicted by the model for 2006. In the models proposed by Aira et al. (2008), rainfall and maximum temperature proved to have the best predictive capacity at the opposite of our results. Li and Kendrick (1995) found that minimum temperature was most important in the growing season. Forecasting models display high predictability when the concentration of spores from the previous day or week is introduced into the models. This may be important as it considers the prevailing conditions for growth, sporulation or spore release and dispersion.

Our findings suggest that the daily or weekly spore concentrations of *Alternaria* could be forecasted using the mean concentrations of spore of the same day or week in other years and the previous spore concentrations recorded 1 day or week before and meteorological parameters mainly frequency of the wind third quadrant, maximum wind speed and minimum relative humidity. This latter factor is probably related to *Alternaria* growth and spore production. These results are specific to biogeographic characteristics and climatic conditions of Tetouan.

Conclusion

The seasonal variation of daily spore concentrations of *Alternaria* spore shows two periods of maximum concentrations, the first in spring and the second in summer or autumn. This bimodal peaks pattern is found in the Mediterranean region. *Alternaria* spores are dispersed passively by the wind and found in greatest abundance when temperature rises, relative humidity decreases and wind speed increases.

The daily fluctuation in spore concentrations is positively associated to inland winds from NW. The NE winds blowing from the Mediterranean Sea contributes to lower *Alternaria* counts.

From the results of predictive models using spores and meteorological variables, it was concluded that daily or weekly spore concentrations can be predicted using the mean

concentrations of spore of the same day or week in other years and the previous spore concentrations recorded 1 day or week before and meteorological parameters mainly frequency of the wind third quadrant, maximum wind speed and minimum relative humidity.

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