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Review

Dispersal patterns of *Alternaria conidia* in Spain

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ABSTRACT

Alternaria is a common airborne phytopathogenic fungus that may affect crops in the field or can cause decay of plant products. It can also cause diseases in animals and humans. The study of airborne *Alternaria* conidia is a necessary step for the control and prevention of the agricultural damage they can provoke. The aim of this paper is to contribute to model the presence and levels of *Alternaria* conidia in the air using a logistic regression model. Our study is conducted in 12 monitoring stations in Spain corresponding to three geographic regions with different bio-climatic characteristics, which show three different patterns of *Alternaria* conidia dynamics: a unique main sporulation season from mid spring to autumn in NE Spain, two defined periods (spring and autumn) in SE Spain and a uniform and constant presence in the Canary Islands. Regarding the abundance, NE Spain shows the highest values and the Canary Islands the lowest.

Daily *Alternaria* conidia concentration is positively correlated to daily minimum temperature and daily temperature variation and negatively correlated to daily precipitation. Also, the occurrence of rain in the 3 previous days has a positive effect on *Alternaria* levels. These effects are modelled in this paper by means of logit regression equations. The three equations used apply to the presence of *Alternaria* conidia, and to the exceedance of thresholds of 10 and 30 conidia/m^3. The model is calibrated in the 12 stations using data from years 1995 to 2008 and validated with data from 2009 in 7 stations, showing a reasonable percentage of right prediction (average 78.6%, ranging from 61.3% to 92.5%).

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1. Introduction

The majority of terrestrial organisms that travel long distances use the atmosphere as their transport medium (Dingle, 1996). Reproduction in botanical and fungal organisms is often dependent on their reproductive cells (pollen and spores) being translated through the air. Production, release and transportation are affected by meteorological and environmental factors. But together with the benefit of the reproduction, the presence of pollen and spores in the air at certain levels may contribute to widen plant epidemics caused by phytopathogenic fungi and also elicit problems in animals (humans included).

Then, as said in Gage et al. (1999), a sound understanding of the biological and meteorological interactions that govern the transport of organisms in the atmosphere is a requisite to develop innovative and successful strategies for protecting human and animal health and for sustaining a large number of terrestrial ecosystems.

Alternaria is a widespread opportunistic pathogen and ubiquitous saprophytic fungus (Mayser et al., 2002; Kirk et al., 2001). It causes diseases with serious economic impact on a large variety of crops, e.g. olive, small grain cereals, tobacco, cauliflower, broccoli, pepper, carrot, potato, and fruits like tomato, citrus, melon or apple (Kirk et al., 2001; Logrieco et al., 2003; Thomma, 2003). In Spain, *Alternaria* is responsible for important losses, regularly or sporadically, even when repeated and intense treatments with fungicides are applied in tomato (Tello, 1999), citrus (Vicent et al., 2000), olive fruit (Moral et al., 2008) or potato (Iglesias et al., 2007). *Alternaria* reproductive structures in the anamorphic phase are conidia or asexual, non-motile and wind dispersed spores. These conidia have clinical significance for the production of toxic secondary metabolites involved in osteomyelitis, pulmonary and cutaneous infections, keratomycosis in humans (Singh et al., 1990), cancer in mammals (Thomma, 2003), phaeohyphomycotic infection in cats (Miller, 2009) and horses (Genovese et al., 2001) and mycotoxicoses in farms (Gruber-Schley and Thalmann, 1988), between others. *Alternaria* is also commonly related to respiratory allergic diseases, through the activation of IgE-mediated antibodies producing rhinitis, asthma and atopic dermatitis (D'Amato et al., 1997; Pereira et al., 2006; Bartra et al., 2009) and in food allergies in humans and animals (Barkai-Golan, 2008).

Since the 1990s, several studies have proved that there is a relationship between airborne pollen concentration and fruit production of plants, such as *Olea* (Galán et al., 2008; Forniaciari et al., 2005; Belmonte et al., 2000), *Vitis vinifera* (Cunha et al., 2003), *Quercus* (Garcia-Mozo et al., 2007), or *Corylus avellana* (Lletjos et al., 1993). Alike, there should be an association between airborne *Alternaria* conidia concentration and agricultural damage as published by Viljanen-Rollinson et al. (2007) and Tomassetti et al. (2009).

Mathematical modelling of crop disease is a rapidly expanding discipline within plant pathology (Van Maanen and Xu, 2003). In the last years, different attempts have been made to model the presence and concentration of *Alternaria* conidia from the meteorological forecasts. This includes three different approaches: (a) applying the current models of atmospheric dispersion to *Alternaria* conidia, (b) using artificial neural networks to predict the mean daily concentration, and (c) statistical analysis.

In the first set, researchers have used either Lagrangian models, such as the HYSPLIT (Isard et al., 2005) and the CALPUFF (Pfender et al., 2006) or Eulerian models (McCartney and West, 2007) to investigate the atmospheric trajectories. In the second set, the artificial neural networks proposed by Grinn-Gofroń and Strzelczak (2008) and Tomassetti et al. (2009) use, besides temperature and rainfall, other meteorological variables like relative humidity, atmospheric pressure or wind velocity. Regarding the third approach, although time series analysis has been also used (Damialis and Gioulekas, 2006), most of the statistical analysis of spore data is based on a correlation approach, in which, due to the nonnormality of the distribution, the Spearman rank correlation is favored (Sánchez Reyes et al., 2009; Aira et al., 2008; Díez Herrero et al., 2006; Rodríguez-Rajo et al., 2005; Sabariego et al., 2000, 2004; Stennett and Beggs, 2004; Fernández et al., 1998; Mitakakis et al., 1997). Nevertheless, linear correlation and regression have also been used (Grinn-Gofroń and Rapiejko, 2009; Erkara et al., 2008; Sousa et al., 2007; Angulo-Romero et al., 1999; Herrero et al., 1996).

We try here a different approach, based on a logistic regression model, for a minimal set of meteorological variables. The advantage over neural network predictive systems is that, since our model is parametric, statistical testing of the parameter estimates can provide evidence of the effect of the different meteorological variables in the presence of conidia in the atmosphere. On the other hand, the logit approach allows us to skip modeling the distribution of the spore concentration.

The aim of this study is to examine the aerobiological dynamics of *Alternaria* conidia at 12 Spanish localities with diverse environmental conditions and the relationship between the conidia levels and some meteorological factors. First, we describe the airborne behavior of *Alternaria* conidia by means of the main aerobiological parameters and graphs showing the dynamics along the year. Second, we provide three models that may help in the prevention and management of plant and animal disease epidemics, reporting their sensitivity and specificity. Finally, we validate these models with data from year 2009, not used in their calibration.

2. Materials and methods

2.1. Study area

In this paper we analyse *Alternaria* data from 12 Spanish locations (Fig. 1). Ten of these localities are in the Iberian Peninsula under Mediterranean influence, 8 in Catalonia and 2 in Andalusia, sharing warm winters, hot summers and rain episodes, especially in fall, followed by spring and winter. The other 2 localities are in the Atlantic insular area of Spain, the Canary Islands, and thus have an Atlantic climate, with mild temperatures all over the year and irregular rains, usually from October to April. In all the localities, the summer is dry. However, there is a wide diversity of local flora due to a varied geomorphology and distance to the sea.

Catalonia is localized in the North-East of the Iberian Peninsula (Fig. 1). According to Allue (1990), the Catalan stations lie in four phytoclimatic regions. Vielha, the northern station, which is placed in a Pyrenean valley, shows the coldest and rainiest conditions (9.9 °C of mean annual temperature and 899 mm of annual rainfall). Bellaterra, Girona and Manresa can be described as fresh (13.6–15.2 °C) and humid (594–740 mm) and the corresponding phytoclimate is Fresh-Continental Oriental-semihumid. Barcelona, Tarragona and Roquetes-Tortosa, in the littoral and the southern part of Catalonia, are hotter and dryer than the previous ones and correspond to the Fresh-Tethyc-semiarid climate. Lleida, at middle latitude of the geographic area and inland, has a more extreme (15.1 °C, 385 mm) climate, which is classified as Fresh-Transitional-semiarid climate (Table 1).

The Andalusian cities of Granada and Almería are located in the South-East of the Iberian Peninsula (Fig. 1). Granada situated inland and at a certain altitude, has a fresher (mean annual temperature 15.6 °C) and rainy climate (annual rainfall 448 mm) than Almería (17.9 °C, 231 mm). Both climates are hotter and dryer than the Catalan ones. According to Allue (1990) in these localities the corresponding phytoclimates are: Fresh-Continental Occidental-semiarid (Granada) and Subtropical-Continental Oriental-semiarid

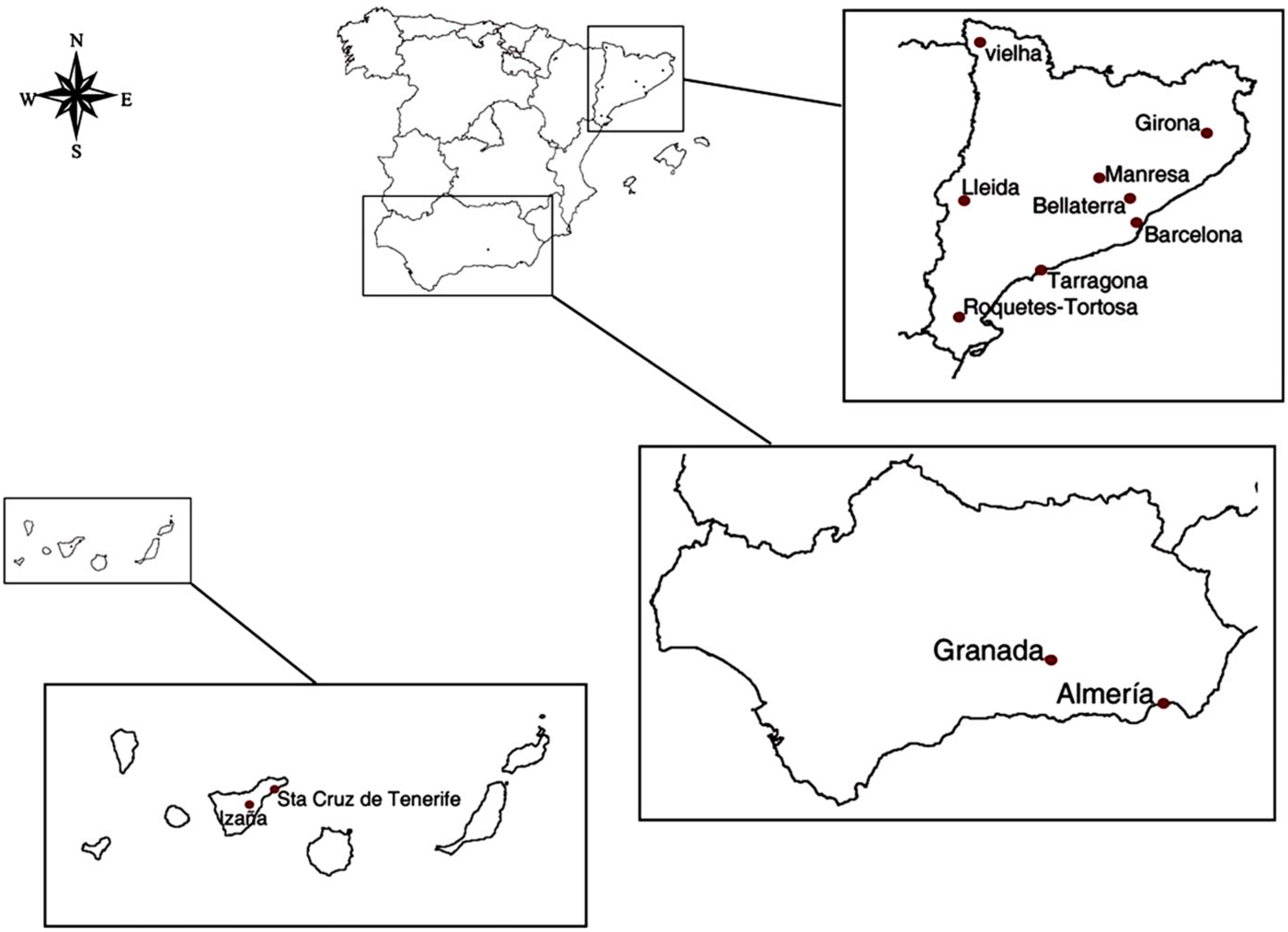

Fig. 1. Geographical situation of the aerobiological stations within the study territory.

climate (Almería). Santa Cruz de Tenerife and Izaña are in the Tenerife Island from the Canary Archipelago, the southernmost and westernmost Spanish territories, located in the Atlantic Ocean (Fig. 1). Santa Cruz, lying by the sea, has a very mild and dry climate (20.8 °C and 251 mm) while Izaña, at 2367 m.a.s.l. in the Teide mountain, is much colder and rainy (9.4 °C and 464 mm). In this Atlantic area, the trade winds provide enhanced humidity. Santa Cruz de Tenerife is classified by Allue (Allue, 1990) as Subtropical-Atlantic semiarid climate and Izaña as Fresh-Continental Oriental semiarid phytoclimate.

Together with the annual values used to characterize the climate and to evidence the gradient of increasing temperature and decreasing rainfall from North to South, it is also important to consider the distribution of these parameters along the year. From this point of view, the most remarkable aspects are: the mountain localities (Izaña and Vielha) and the inland cities (Lleida, Manresa and Granada) register higher temperature oscillations, with cold winters and warm summers, than the other peninsular sites. Santa Cruz de Tenerife does not show important temperature differences along the year. Regarding precipitations, while the coast stations

Table 1
Geographical characteristics of the aerobiological stations and corresponding climatic characteristics of the area.

Geographical characteristics				Climatic characteristics (Allue 1990)		
Sampling stations	Environment	Altitude (m. a.s.l.)	Geographical Coordinates	Tmean (°C)	Prec (mm)	Phytoclimates
Barcelona	Urban	93	41°24′N, 02°09′E	16.4	593	Fresh-Tethyc-semiarid
Bellaterra	Rural/Urban	245	41°34′N, 02°06′E	15.2	594	Fresh-Continental Oriental-semihumid
Girona	Urban	98	41°59′N, 02°50′E	15.0	740	Fresh-Continental Oriental-semihumid
Lleida	Rural	202	41°37′N, 00°35′E	15.1	385	Fresh-Transitional-semiarid
Manresa	Rural/Urban	291	41°43′N, 01°49′E	13.6	619	Fresh-Continental Oriental-semihumid
Tarragona	Urban	44	41°07′N, 01°15′E	15.8	478	Fresh-Tethyc-semiarid
Roquetes-Tortosa	Rural/Urban	14	40°49′N, 00°30′E	16.8	576	Fresh-Tethyc-semiarid
Vielha	Rural	974	42°42′N, 00°47′E	9.9	899	Fresh-Continental Oriental-humid
Almería	Urban	23	36°50′N, 02°27′W	17.9	231	Subtropical-Continental Oriental-semiarid
Granada	Urban	685	37°11′N, 03°35′W	15.6	448	Fresh-Continental Occidental-semiarid
Izaña	Rural	2367	28°18′N, 16°30′W	9.4	464	Fresh-Atlantic-semiarid
Sta Cruz Tenerife	Urban	37	28°28′N, 16°15′W	20.8	251	Subtropical-Atlantic-semiarid

Tmean: mean annual temperature; Prec: annual rainfall.

Table 2
Variables involved in the prediction models.

Variable	Type	Description
Dependent		
alte1	Dummy	1 if *Alternaria* concentration is nonzero, 0 otherwise
alte10	Dummy	1 if *Alternaria* concentration exceeds 10 conidia/m^3, 0 otherwise
alte30	Dummy	1 if *Alternaria* concentration exceeds 30 conidia/m^3, 0 otherwise
Independent		
Tmin	Continuous	Daily minimum temperature, Celsius
Tran	Continuous	Daily difference maximum minus minimum daily temperature, Celsius
Prec	Dummy	1 if there is rainfall the same day, 0 otherwise
Precpast	Dummy	1 if there was rainfall 2 or 3 days ago

(Girona, Bellaterra, Barcelona, Tarragona, Roquetes-Tortosa and Almería) show a regime with more important rainfall episodes during autumn, in the inland peninsular stations the rain prevail in spring. In the Canary stations the major rainfalls are observed during winter and autumn and the trade winds provide extra humidity all the year long. In addition, all the stations show an important drought period in summer, whose importance increases from North to South.

2.2. Meteorological data

The raw meteorological variables considered in this study were maximum and minimum daily temperatures and daily rainfall. More details are given in Section 2.4 and Table 2. The data for the Catalan stations have been provided by the Agencia Estatal de Meteorología (AEMET) (Barcelona until 2001; Bellaterra-Sabadell until 1999; Girona, Lleida and Tarragona until 2000; Manresa and Roquetes-Tortosa) and Servei Meteorològic de Catalunya (Meteocat) (Barcelona since 2002; Bellaterra-Sabadell since 2000; Girona, Lleida and Tarragona since 2001 and Vielha); for the Andalusian stations (Granada and Almería) Red de Alerta e Información Fitosanitaria (RAIF Meteorological Network) provided the data, and for the Canary island stations (Santa Cruz de Tenerife and Izaña) the provider was the Agencia Estatal de Meteorología (AEMET).

2.3. Aerobiological sampling method

The samples were obtained with a Hirst sampler (Hirst, 1952) and analysed following the standard methodology proposed by the Spanish Aerobiology Network (Red Española de Aerobiología, REA) (Galán et al., 2007). The spore collectors are placed on top roof of buildings, between 25 and 30 m.a.g.l. in all cases except in Manresa, where since 2002 the sampler is placed at a maximum of 5 m.a.g.l. The concentrations were expressed as mean daily number of conidia per cubic metre of air (conidia/m^3). The stations provided different number of yearly periods comprised between 1995 and 2008, depending on the existing data: 14 yearly series for Barcelona and Bellaterra, 9 for Girona, 8 for Manresa and Tarragona, 7 for Lleida, 5 for Granada and Vielha, 4 for Almería and Tenerife and 3 for Izaña and Roquetes-Tortosa. Since Izaña is a station at 2367 m.a.s.l., the sampling period per year was from May to October.

2.4. Statistical methods

In this paper, the statistical analysis is based on the logit regression model. Logit models are described in many Statistics textbooks, the standard reference being Hosmer and Lemeshow (2000), where the interpretation of regression coefficients in terms of odds ratios is explained (Chapter 3). The regression equations were fitted with the logit command of Stata 10 (Stata, 2007) based on maximum likelihood estimation. Multicollinearity diagnostics were obtained with the collin command. A Stata based introduction to regression with discrete dependent variables can be found in Long and Freese (2006).

Besides choosing the right predictors, the main drawback of common regression models comes from the statistical distribution of the variables included in the model equation, which is hardly compatible with the assumptions implicitly made when fitting linear regression models. On the left hand side of the regression equation, the main traits of the distribution of the concentration that we wish to predict are: (a) a relevant amount of zeros (between 3% and 56% in our case, as shown in Table 4), (b) no negative values, and (c) extremely high values. On the right hand side, the inclusion of rainfall, which may be expected to be a relevant predictor, calls for a special treatment, since a linear dependence of conidia concentration on rainfall is hardly imaginable.

We reduce here the difficulties of managing conidia concentration and rainfall by transforming them into discrete variables. More specifically, we use dummy variables to denote the presence of *Alternaria* conidia, the exceedance of a threshold conidia level, the presence of rain on the same day and on previous days.

Having a dummy as the dependent variable, it is appropriate to use a logit regression model, based on an equation of the type

$$\log\left[\frac{p}{1-p}\right] = \beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k$$

in which $X_1, \ldots, X_k$ are the predictors or explanatory variables, and p is the proportion in which a certain outcome is observed. The left hand side is usually written as logit(p). For instance, the predictors

Table 3
Sites and period under study and main meteorological parameters of the period. Tmean (mean annual temperature), Tmin (mean annual minimum temperature), Tran (mean annual thermal oscillation), Prec (mean annual rainfall).

Sites	Years sampled	Days with data (%)	Tmean (°C)	Tmin (°C)	Tran (°C)	Prec (mm)	Rainy days (%)
Barcelona	1995–2008	98.9	15.9	11.9	7.9	566	21.6
Bellaterra	1995–2008	98.6	16.3	10.7	10.6	505	27.0
Girona	2000–2008	91.8	16.3	10.3	12.0	653	25.7
Lleida	2002–2008	92.3	12.5	8.9	12.1	321	29.6
Manresa	2000, 2002–2008	98.6	14.9	8.7	12.4	584	20.0
Tarragona	2000, 2002–2008	95.9	17.1	12.3	9.6	580	22.8
Roquetes-Tortosa	2006–2008	97.8	18.1	12.6	11.1	556	29.0
Vielha	2004–2008	91.2	10.4	4.5	11.8	833	40.7
Almería	1998–2001	100	19.2	15.2	8.2	162	9.9
Granada	2002–2006	100	15.9	8.9	14.0	304	21.8
Izaña[a]	2006–2008	45.7[b]	10.3	6.6	7.4	419	13.6
Sta Cruz Tenerife	2005–2008	97.8	21.6	18.6	5.9	276	15.1

[a] The study is from May to October.
[b] Related to the whole year.

Table 4
Summary statistics for *Alternaria:* percentages of days in the study period with concentrations accomplishing the indicated thresholds.

Sites	Mean annual index (conidia)	Daily concentrations		% days			
		Mean (conidia/m^3)	Absolute Maximum (conidia/m^3)	Without	<10 conidia/m^3	10–30 conidia/m^3	>30 conidia/m^3
Barcelona	8942	25	471	19	28	26	28
Bellaterra	12,271	34	927	12	24	26	37
Girona	12,511	37	633	15	27	20	34
Lleida	31,610	94	1196	3	11	22	63
Manresa	21,770	60	1388	7	20	20	47
Tarragona	10,336	30	1506	12	29	30	28
Roquetes-Tortosa	11,687	33	554	10	28	27	35
Vielha	1443	4	118	56	30	12	2
Almería	5952	16	229	4	46	37	13
Granada	6292	17	226	14	39	29	17
Izaña[a]	527	3	39	40	40	6	0
Sta C. Tenerife	1533	4	199	44	45	9	1

[a] The study period is from May to October.

may be the minimum daily temperature, the daily thermal oscillation or range, a dummy variable indicating whether it rains or not (rain = 1, no rain = 0) in the same day and another dummy indicating rain on the previous days. *p* can be the probability of catching *Alternaria* conidia in our sampling device.

The dependent variables are dummy variables related to *Alternaria* conidia concentrations, as explained in Table 2, while the independent variables are related to daily temperature and precipitation parameters. Temperatures come in Celsius degrees, while precipitation enters the equation in the form of dummy variables. As predictors, we use the minimum daily temperature [Tmin], the daily temperature range or oscillation [Tran] (difference between maximum and minimum temperature), the occurrence of rainfall in the same day [Prec] and the occurrence of rainfall in the 3 last days [Precpast].

We have used three logit regression equations [alte1, alte10, alte30]. Whereas the first model [alte1] predicts the presence of *Alternaria* conidia in the atmosphere, [alte10] informs on exceeding 10 conidia/m^3 and [alte30] on exceeding 30 conidia/m^3. The thresholds of spore infections in crops are not known. In the absence of bibliographical references on levels of airborne *Alternaria* and degree of affectation on cultures, we have decided to base the thresholds on the aerobiological experience of the authors. Later, we have seen that for other fungal species (*Botrytis*) thresholds (Carise et al., 2008; Kennedy and Wakeham, 2008) coincident to ours are cited.

Although logit regression models are not optimal for real-time forecasting, they can sometimes be used for this purpose, after specifying a cut point that allows transforming the probabilities predicted into positive/negative predictions. To illustrate our models, we have done so, reporting the sensitivity and specificity, quite popular in Biostatistics, especially in Epidemiology (Campbell and Machin, 2007), as measures of the predictive power. The sensitivity is the percentage of true positives with respect to the total observed positives, while the specificity is that of true negatives with respect to the total of observed negatives.

Finally, we have validated this model with data from year 2009 not included in the elaboration of the equation and using Stata 10 (Stata, 2007).

3. Results

3.1. Meteorological study

The 12 sampling sites displayed different meteorological conditions during the study period (Table 3). Vielha, at a high mountain level, registered a mean annual minimum temperature [Tmin] below 5 °C, whereas in the coastal zones it is higher than 11 °C. On the other hand, the major temperature oscillations [Tran], with values ranging between 14.0 °C in Granada and 12.0 °C in Girona, were found in the inland stations. The rainfalls also presented important differences. Table 3 shows that in mountain zones, such as Vielha, the percentage of annual rainy days is higher than 40% whereas in the driest zones (Almería, Sta Cruz de Tenerife) this percentage does not exceed 15%.

3.2. Aerobiological study

The summary parameters for *Alternaria* in the studied sites are shown in Table 4. The lowest values correspond to the mountain zones: Vielha, with a mean daily average of 4 conidia/m^3, a mean daily maximum of 118 conidia/m^3 and 56% of days without *Alternaria* conidia, and Izaña (period May–October) with 3 conidia/m^3, a maximum of 39 conidia/m^3 and 40% of the days with no presence. Santa Cruz de Tenerife also shows very low values, followed by Almería and Granada. All these localities show a long drought period, lasting at least from May to mid October. In Catalunya, where drought lasts maximum since June to mid September, values are much higher, ranging from the mean daily average of 25 conidia/m^3, a mean daily maximum of 471 conidia/m^3 and 19% of days without conidia in Barcelona to the mean daily average of 94 conidia/m^3, a mean daily maximum of 1196 conidia/m^3 and 3% of days free of *Alternaria* conidia presence in Lleida.

The differences in the amount of Alternaria conidia in the air are also evidenced in Fig. 2, which shows the annual dynamics of the mean weekly conidia concentrations for the study period by means of a graph for each locality. While in Catalonia the atmospheric *Alternaria* conidia levels present one period of maximum concentration beginning at the end of spring, peaking in summer and decreasing during autumn, in Granada and Almería (specially in the first one) there are two main spore periods, the first during spring and the second in autumn, possibly resulting from the humidity provided by autumn rains. In Canary Islands (Santa Cruz de Tenerife and Izaña), the concentration is more uniform and constant.

In the same way, Fig. 2 shows that in the majority of Catalan stations, *Alternaria* weekly averages rarely exceed 200 conidia/m^3. In Vielha, the coldest Catalan station, values do not exceed 50 conidia/m^3, as also happens in the Canary Islands. On the other hand, Granada and Almeria presented average weekly values lower than 100 conidia/m^3.

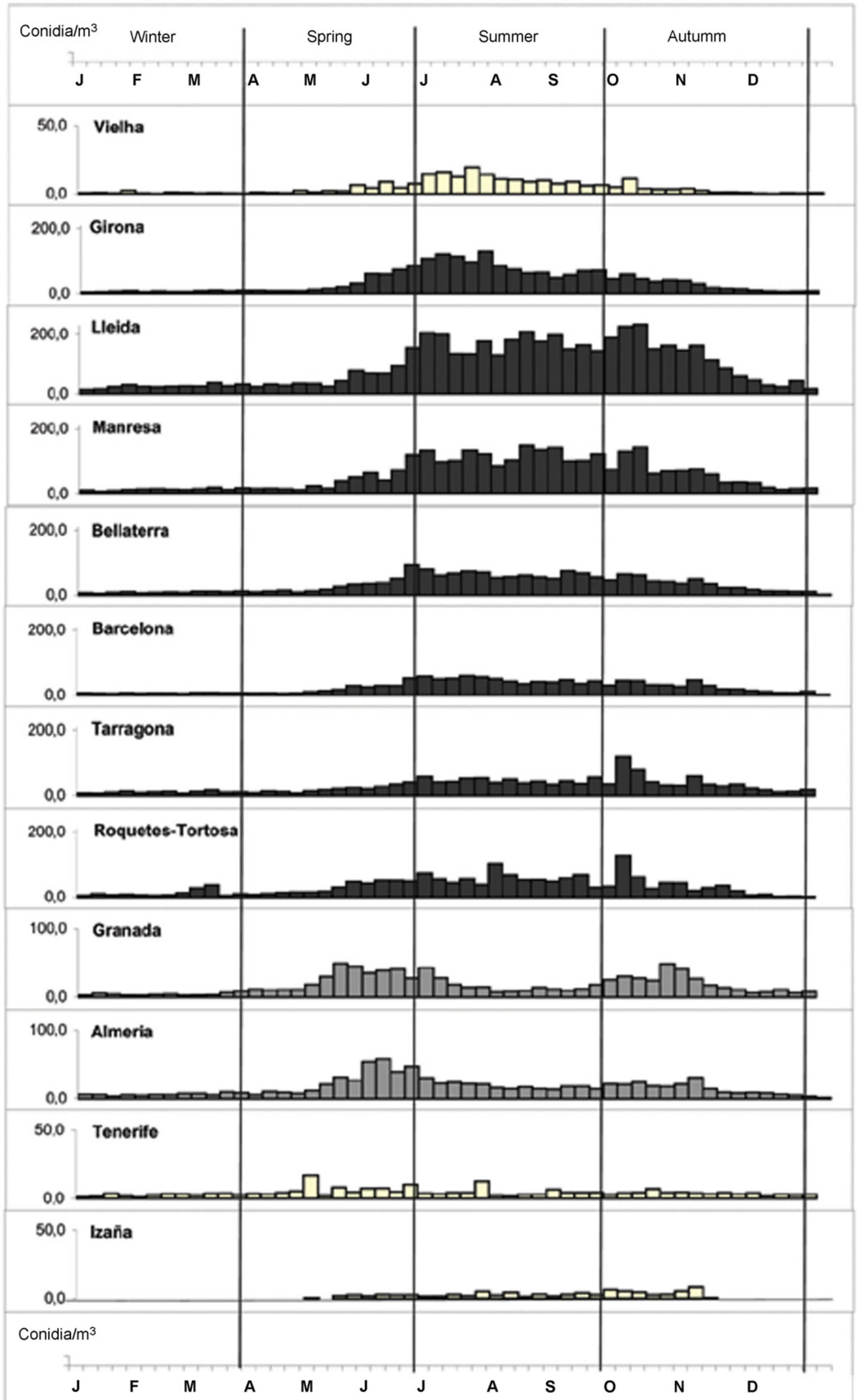

Fig. 2. Yearly dynamics of *Alternaria conidia* in the sampling stations (mean weekly concentrations along the year during the study period).

Table 5
Results of the logit regression analysis. Tmin (minimum temperature), Tran (thermal oscillation), Prec (rainfall in the day), Precpast (rainfall in the 3 previous days).

	alte1	alte10	alte30		alte1	alte10	alte30
Barcelona				**Roquetes Tortosa**			
Tmin	0.174***	0.190***	0.176***	Tmin	0.276***	0.278***	0.232***
Tran	0.136***	0.105***	0.061***	Tran	0.173***	0.130***	0.129***
Prec	−0.336***	−0.297***	−0.268**	Prec	−0.037	0.596**	−0.232
Precpast	0.196*	0.336***	0.404***	Precpast	−0.136	0.110	0.380*
Ct. β_0	−1.325***	−2.957***	−3.848***	Ct. β_0	−2.014***	−3.996***	−5.389***
Sensitivity	97.2%	74.5%	33.9%	Sensitivity	98.9%	84.2%	61.3%
Specificity	10.7%	72.1%	89.6%	Specificity	14.6%	71.3%	84.1%
Bellaterra				**Vielha**			
Tmin	0.180***	0.225***	0.228***	Tmin	0.204***	0.275***	0.198***
Tran	0.134***	0.136***	0.108***	Tran	0.120***	0.089***	0.109***
Prec	−0.339**	−0.390***	−0.196*	Prec	0.053	−0.388*	−0.570
Precpast	0.061	0.263***	0.581***	Precpast	0.098	0.381*	0.150
Ct. β_0	−0.864***	−3.073***	−4.574***	Ct. β_0	−2.737***	−5.790***	−6.647***
Sensitivity	99.7%	83.2%	64.6%	Sensitivity	74.1%	23.0%	0%
Specificity	2.5%	63.0%	84.1%	Specificity	79.2%	96.9%	100%
Girona				**Almería**			
Tmin	0.209***	0.254***	0.274***	Tmin	0.290***	.239***	0.148***
Tran	0.132***	0.135***	0.143***	Tran	0.192*	0.109***	0.120***
Prec	−0.596***	−0.551***	−0.080	Prec	−1.216**	−0.777**	−0.815*
Precpast	−0.200	0.223***	0.603***	Precpast	−0.758*	0.163	0.690**
Ct. β_0	−1.119***	−3.574***	−5.784***	Ct. β_0	−1.325	−4.417***	−5.357***
Sensitivity	98.2%	81.5%	69.6%	Sensitivity	100%	71.5%	0.5%
Specificity	9.9%	74.4%	85.4%	Specificity	0.0%	77.6%	99.8%
Lleida				**Granada**			
Tmin	0.087***	0.139***	0.175***	Tmin	0.136***	.117***	0.102***
Tran	0.250***	0.221***	0.197***	Tran	0.116***	0.057***	0.044**
Prec	−0.338	−0.446**	−0.082	Prec	−0.372*	−0.388**	−0.246
Precpast	−0.258	0.135	0.366**	Precpast	0.014	0.325**	0.891***
Ct. β_0	0.721	−1.426***	−3.318***	Ct. β_0	−0.532	−2.003***	−3.473***
Sensitivity	100%	97.6%	85.4%	Sensitivity	99.7%	62.4%	0.3%
Specificity	0.0%	24.2%	65.0%	Specificity	3.5%	70.5%	99.9%
Manresa				**Izaña**			
Tmin	0.128***	0.184***	0.211***	Tmin	0.049*	−0.032	
Tran	0.117***	0.119***	0.124***	Tran	−0.017	0.168	
Prec	−0.482**	−0.662***	−0.585***	Prec	0.569	0.659	
Precpast	0.228	0.293**	0.532***	Precpast	0.274	0.257	
Ct. β_0	0.512	−1.621***	−3.578***	Ct. β_0	−0.334	−3.642**	
Sensitivity	100%	90.0%	72.6%	Sensitivity	70.7%	0.0%	
Specificity	0.0%	42.5%	78.6%	Specificity	32.9%	100%	
Tarragona				**Sta Cruz Tenerife**			
Tmin	0.149***	0.170***	0.158***	Tmin	0.104***	0.098**	
Tran	0.161***	0.165***	0.158***	Tran	0.162***	0.275***	
Prec	−0.331*	0.010	0.033	Prec	0.154	−0.224	
Precpast	−0.073	−0.002	0.369***	Precpast	0.497***	−0.013	
Ct. β_0	−0.954**	−3.221***	−4.687***	Ct. β_0	−2.775***	−5.643***	
Sensitivity	99.8%	76.3%	29.3%	Sensitivity	79.5%	1.3%	
Specificity	1.7%	60.6%	90.8%	Specificity	35.0%	100%	

* $0.01 < P < 0.05$.
** $0.001 < P < 0.01$.
*** $P < 0.001$.

3.3. Data analysis

Table 5 shows the estimates of the coefficients of the explanatory variables for the models [alte1], [alte10] and [alte30] as well as the resulting sensitivity and specificity. To be sure that the potential correlation between meteorological variables cannot raise a multicollinearity problem leading to the unreliability of the coefficient estimates, variation inflation factors (VIF) for all the regressors were calculated. The values obtained fall between 1 and 1.5, dismissing so the concerns about multicollinearity. In [alte1] we observe a significant, positive effect of both Tmin and Tran, with the only exception of Tran in Izaña. The practical meaning of these effects can be assessed after a simple calculation based on odds ratios. In Barcelona, where the effect of the minimum daily temperature is 0.174 (Table 5), an increase of one degree leads to an odds ratio OR = exp(0.174) = 1.19. This implies, for a number of days with *Alternaria* conidia of 80% (as in Barcelona, see Table 4), that the odds increases from 4 to 4.76, which corresponds to an increase of 2.6% in the number of days with *Alternaria*.

The rainfall has a negative effect on the occurrence of *Alternaria* conidia in the same day (Table 5). Leaving aside the Canary Islands, this effect, though nonsignificant in some Catalan sites (Lleida, Roquetes-Tortosa and Vielha), becomes significant if we drop Tran from the equation. This can be easily explained by the negative association between rain and daily temperature range: the within-day variation of temperature is lower on rainy days. The assessment of the rainfall effect can be done as above, but now Prec is a dummy variable. As an example, in Barcelona, the coefficient is −0.336 (Table 5), hence OR = exp(−0.336), which means that changing from Prec = 0 (no rain) to Prec = 1 (rain) decreases the incidence of *Alternaria* conidia from 80% to 74.1%.

Regarding the occurrence of rainfall in the 3 previous days [Precpast], the effect is to cause a decrease in some stations (significant in Izaña, Roquetes-Tortosa and Almería and nonsignificant in Girona, Lleida and Manresa) and an increase in others (significant in Barcelona and Santa Cruz de Tenerife and nonsignificant in Bellaterra, Tarragona, Vielha and Granada).

Since a logistic model does not predict individual values but probabilities, in order to assess its predictive power we must transform each probability into a prediction of whether the event predicted is going to occur or not. To illustrate the presentation of our results, we have done this in the simplest way, assigning a positive prediction to those situations for which the predicted probability *p* exceeds 0.5 and a negative one to those where it hasn't. Then, we have matched the prediction to the actual data using the sensitivity and specificity. [Alte1] shows the best sensitivity (agreement on presence of conidia in the air), as expected by the simplicity of cases to predict. The only exceptions are the localities with high percentage of days without conidia (Vielha, Santa Cruz de Tenerife and Izaña), where, as expected also, the specificity (agreement on no conidia in the air) is the highest measured. Since the percentage of days in which *Alternaria* conidia are observed is very high in most of the sites (Table 4), the specificity is quite low in the [alte1] column, unless the cut point 0.5 is changed.

In [alte10], the values obtained show that it is possible to derive a reasonable forecasting of the exceedance of threshold levels from these simple models even without adjusting them. For instance, in Barcelona, Stata reports for 10 conidia/m^3 [alte10] 2616 days with positive prediction ([alte10] = 1) and 2347 with negative prediction ([alte10] = 0). When comparing these results with actual data, 1949 of the positive predictions were right (true positives, and thus the sensitivity is 74.5% as shown in Table 5) and 667 were wrong (false positives). Also, 2347 of the negative predictions were right (true negatives, and thus the specificity is 72.1% as shown in Table 5) and 655 were wrong (false negatives).

In general, the values reported are quite satisfactory in many cases, with sensitivity values between 97.6% for Lleida and 62.4% for Granada. However, in some sites, such as Vielha, the model does not include enough explanatory variables for the predictions to be reliable (the results show a 23% of sensitivity). Regarding the specificity obtained, it ranges from 97% to 100% in Vielha, Santa Cruz de Tenerife and Izaña to 63–61% in Bellaterra and Tarragona, leaving aside the localities with the highest conidia content (Lleida and Manresa).

Finally, the high levels of *Alternaria* conidia [alte30] show practically the same results that the model with more than 10 conidia/m^3 [alte10], with the only exception of Tenerife stations. While the temperature has a positive effect, that of the rainfall is negative. The variable Precpast is more significant with a positive effect, stronger in Barcelona, Bellaterra, Girona, Manresa, Almería and Granada. The [alte30] model shows better specificity (over 79%, with the only exception of Vielha 65%) than sensitivity (over 61%, with the exceptions of Barcelona (34%) and Tarragona (29%)). As a rule, the sensitivity in the localities where the mean Annual conidia index falls below 10,000 conidia and the mean daily conidia concentration below 30 conidia/m^3 (Table 4) shows [alte30] nonsignificant values (0.5% Almería, 0.3% Granada, and 0% Vielha). Values as high as 30 conidia/m^3 are rarely attained in Tenerife stations.

Table 6
Validation results obtained using the aerobiological and meteorological data series from year 2009.

	[alte1]	[alte10]	[alte30]
Barcelona	83.7%	77.1%	72.4%
Bellaterra	89.2%	76.8%	76.8%
Girona	82.9%	84.3%	79.8%
Lleida	92.5%	82.6%	72.1%
Manresa	92.0%	78.5%	78.5%
Tarragona	63.3%	61.3%	70.4%
Vielha	61.9%	78.7%	94.8%

3.4. Validation

Finally we have used data from year 2009 for the validation of the models obtained. Inputting in the equations fitted to the data of the calibration period (1995–2008) the meteorological data of year 2009, we have used the rule described in the preceding paragraph to assign either 1 or 0 to alte1, alte10 and alte30, comparing the predicted to the observed values and reporting the percentage of coincidence in Table 6.

4. Discussion

Mediterranean basin is a large geographical region with a diversified agricultural system. On the host plants, pathogenic fungi may induce plant disease (Logrieco et al., 2003). In general, *Alternaria* species are foliar pathogens that cause destruction of host tissues through the reduction of photosynthetic potential (Thomma, 2003).

The present study contributes to the knowledge of the factors affecting the dynamics of airborne *Alternaria* conidia at 12 Spanish localities. It attempts to contribute to the comprehension of the dispersion of these reproductive elements, and help the prevention and management of plant disease epidemics by applying the fight against the fungus at the best moment.

In this paper we show three different dynamic patterns (Table 4, Fig. 2). In North-East Spain (Catalonia), levels of *Alternaria* are higher than in the other studied regions and there is a unique sporulation period which peak during summer. This dynamic has been also observed in other cities in the North and the Centre of Spain (i.e. Vigo and Ourense in Rodríguez-Rajo et al., 2005, León in Fernández et al., 1998, Valladolid in Sánchez Reyes et al., 2009, Palencia in Herrero et al., 1996, Madrid in Díez Herrero et al., 2006) and in some European cities (i.e. the Polish localities of Warszawa, Szcein or Olztyn in Grinn-Gofroń and Rapiejko, 2009, Eskisehir in Turkey in Erkara et al., 2008 and Stockholm in Sweden in Hjelmroos, 1993). In the southern Iberian localities studied (Granada and Almeria) *Alternaria* daily levels do not exceed 230 conidia/m^3 and show two defined periods (spring and autumn), as stated also in other Andalusian localities as Córdoba by Angulo-Romero et al. (1999), Granada by Sabariego et al. (2000) and Almería by Sabariego et al. (2004) and other localities such as in Sydney (Australia) by Stennett and Beggs (2004). In the Canary Islands, levels are very low and the dynamic pattern is more uniform and constant. Although one of the Hirst collectors in this study (Manresa) was placed at a lower height above ground level than the others, the results obtained show no differences with the results in the other localities of the Catalan region. We assume, as in Rantio-Lehtimäki et al. (1991), that sampling height may affect the quantity of conidia but that counts are correlated.

A phytoclimate is an ecological phenomenon represented by a field experience between plants and climate. It testifies the apparent effect of climate on the distribution of the presence and abundance of the plants on a territory (Garbolino et al., 2008). In the 12 localities of this study, eight phytoclimatic regions are catalogued (Table 1). According to our results (Table 4, Fig. 2), the phytoclimate that best fits with *Alternaria* is the Fresh-Transitional-semiarid (Lleida), followed by the Fresh-Continental Oriental-semihumid (Girona, Manresa and Bellaterra) and the Fresh-Tethyc-semiarid (Barcelona, Tarragona and Roquetes-Tortosa). The phytoclimate with less *Alternaria* conidia is the Fresh-Continental Oriental-humid (Vielha), followed by the Subtropical and Fresh Atlantic-semiarid types. Within each phytoclimatic region, the airborne dynamics of the conidia shows a similar trend, although modelled by the weather parameters and by the proximity to cultivated lands. This study shows, as expected, that the amounts of *Alternaria* conidia (Table 4) collected in the sampling stations with rural or rural/urban environments (Table 2) were greater than those of urban stations. Therefore the airborne fungal spectrum of a given area is dependent on its meteorological factors and on the vegetation of the region.

The strong weather dependency of plant pathogens such as *Alternaria* has been reported in several studies (Corden and Millington, 2001; Friesen et al., 2001; Corden et al., 2003). Our results, showing that daily *Alternaria* conidia concentration has a positive correlation with daily minimum temperature and a negative correlation with daily precipitation, agrees with the findings in other studies (Pereira et al., 2006; Sánchez Reyes et al., 2009; Rodríguez-Rajo et al., 2005; Stennett and Beggs, 2004; Fernández et al., 1998; Mitakakis et al., 1997; Angulo-Romero et al., 1999; Herrero et al., 1996; Oliveira et al., 2009). In addition to the fact that the rainfall in the same day provokes a decrease in the number of airborne particles, this paper shows that the rain occurring in the 3 previous days induces the opposite effect, enhancing the increase of *Alternaria*.

We propose three logistic regression models based on very simple meteorological parameters that provide rather good estimates on the probability of the presence or absence of *Alternaria* conidia [alte1], and the overcome of a threshold of 10 conidia/m^3 [alte10] and a threshold of 30 conidia/m^3 [alte30] and we evaluate them using the sensitivity/specificity concepts. In the localities with continuous values of conidia in the atmosphere, the sensitivity is quite satisfactory, but the specificity is the opposite. For example, in Almeria, with only 3.6% of the days without conidia (Table 4), the sensitivity in [alte1] is 100% and specificity of 0% (Table 5). However, in this station, concentrations of *Alternaria* higher than 30 conidia/m^3 are not usual and thus [alte30] presents a sensitivity of 0% and a specificity of 99.8%.

When applying the corresponding equations to data from year 2009 we obtain reasonable percentages of coincidences (Table 6), ranging from 92.5% (Lleida [alte1]) to 61.3% (Tarragona [alte10]). It is also observed that validation is good independently of the number of years used to produce the model (from 14 years for Barcelona and Bellaterra to only 5 years in Vielha).

All this indicates that the logit model is able to describe the conidia emission pattern by means of simple regression equations using basic weather parameters (Tmin, Tran, Prec, Precpast). Unlike other predictive systems, this model is parametric, allowing for statistical testing.

The examples shown in this paper were always referred to annual data but the same methodology can be applied to shorter periods of time (months, weeks, days), producing relevant information on the evolution of conidia concentrations in a near future. This can help to manage in front of predicted episodes of phytopathogenic particles in the air.

5. Conclusions

The more humid and temperate the climate, the more frequent the occurrence of *Alternaria* conidia in the air. Cold (associated to altitude) and drought are the cause of lower *Alternaria* conidia concentrations. The characterization of a territory by means of the Phytoclimate helps to characterize the dynamics of *Alternaria* conidia along the year, although it is modulated by the meteorology. Categorizing the environments as urban or rural, we find that the last show higher conidia contents.

This paper presents a simple model, based on logistic regression, for the occurrence of three levels (1, 10 and 30 conidia/m^3) of *Alternaria* conidia in the air. The model uses four explanatory variables, which can be obtained from data on maximum and minimum temperatures and precipitation. Since forecasts on these meteorological variables are currently available in many websites, this model allows, not just for a description of the statistical dependence between meteorology and conidia presence in the air, but for real forecasts. The simplification of the variables related to the precipitation, which enter the model as dummies, makes this even simpler. The model has been calibrated in 12 sites using data from years 1995 to 2008 and validated with 2009 data from 7 sites, showing a reasonable accuracy.

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