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Environmental drivers of the seasonal exposure to airborne *Alternaria* spores in Spain

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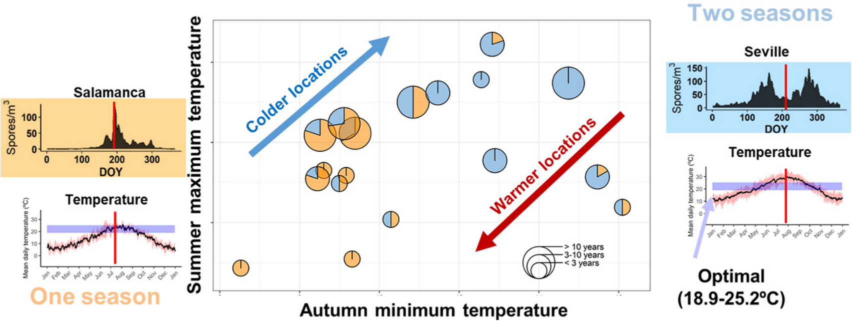
HIGHLIGHTS

- Temperature is the main driving factor for the seasonality of *Alternaria* spores.
- Warmer sampling locations have longer *Alternaria* spore seasons.
- The water availability is also a relevant variable for *Alternaria* spore production.
- Warm summers split in two the *Alternaria* spore season.

GRAPHICAL ABSTRACT



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ABSTRACT

Alternaria conidia have high allergenic potential and they can trigger important respiratory diseases. Due to that and to their extensive detection period, airborne *Alternaria* spores are considered as a relevant airborne allergenic particle. Several studies have been developed in order to predict the human exposure to this aeroallergen and to prevent their negative effects on sensitive population. These studies revealed that some sampling locations usually have just one single *Alternaria* spore season while other locations generally have two seasons within the same year. However, the reasons of these two different seasonal patterns remain unclear. To understand them better, the present study was carried out in order to determine if there are any weather conditions that influence these different behaviours at different sampling locations. With this purpose, the airborne *Alternaria* spore concentrations of 18 sampling locations in a wide range of latitudinal, altitudinal and climate ranges of Spain were studied. The aerobiological samples were obtained by means of Hirst-Type volumetric pollen traps, and the seasonality of the airborne *Alternaria* spores were analysed. The optimal weather conditions for spore production were studied, and the main weather factor affecting *Alternaria* spore seasonality were analysed by means of random forests and regression trees. The results showed that the temperature was the most relevant variable for the *Alternaria* spore dispersion and it influenced both the spore integrals and their seasonality. The water availability was also a very significant variable. Warmer sampling locations generally have a longer period of *Alternaria* spore detection. However, the spore production declines during the summer when the temperatures are extremely warm, what splits the favourable period for *Alternaria* spore production and dispersion into two separate ones, detected as two *Alternaria* spore seasons within the same year.

1. Introduction

Fungal aeroallergens are the cause of important and diverse allergic respiratory diseases (Bongomin et al., 2017; Denning et al., 2014), even during the periods in which allergy related to other airborne particles is not very relevant (Elvira-Rendueles et al., 2019). *Alternaria* conidia are produced by more than 300 species of saprophytic fungi that belong to the Ascomycota phylum (Seifert et al., 2011; Woudenberg et al., 2015). The high allergenic potential of these asexual spores together with the ubiquitous distribution of the fungi species that produce them make *Alternaria* spores a relevant allergenic bioaerosol (Astray et al., 2010; Kustrzeba-Wójcicka et al., 2014).

The prevalence of respiratory allergy diseases due to sensitization to *Alternaria* allergens is estimated to be around the 4.4% (average) in the world and around 6.1% in Europe. In Spain, the rate of sensitization to this aeroallergen ranges from 0.2% to 1.9% depending on the region (Bousquet et al., 2007a, 2007b; Hernandez-Ramirez et al., 2021). Although these rates may seem relatively low, the symptoms that these spores are able to trigger in allergenic population can be quite severe. *Alternaria* spore allergens can produce asthma, bronchitis, rhinitis, eczema and alveolitis in sensitive population (Bush and Prochnau, 2004; Mitakakis et al., 2001a; Randriamanantany et al., 2010). Moreover, they can increase the severity of the symptoms of other respiratory diseases, reaching levels that can even threaten human lives (Apangu et al., 2020; Gabriel et al., 2016; Mitakakis et al., 2001a; Olsen et al., 2020). In order to predict the human exposure to this aeroallergen and to prevent these negative effects on sensitive population, numerous studies have been developed worldwide using different approaches, such as autoregressive models, random forests, time series analysis, artificial neural networks, linear regressions or air mass trajectories (Apangu et al., 2020; Astray et al., 2010; Damialis and Gioulekas, 2007; Grinn-Gofroń et al., 2019; Grinn-Gofroń and Strzelczak, 2008; Katial et al., 1997; Mitakakis et al., 2001b; Olsen et al., 2020).

According to previous studies, the *Alternaria* Main Spore Season (MSS) is usually detected in mid-summer in most central and northern European localities (Grinn-Gofroń et al., 2019; Skjøth et al., 2016). However, the MSS in Southern Europe varies widely among sampling locations: in some of them the MSS happens during late spring while in others, it happens at the beginning of autumn (Almeida et al., 2018; Grinn-Gofroń et al., 2019), or even two separate seasons are detected within the same natural year (one in late spring and another one in early autumn) (Bardei et al., 2016; Infante et al., 1999; Marchesi, 2019; Sabariego et al., 2012). This last behaviour is usually observed in some Mediterranean areas of Spain, Italy or Greece, where it is frequent to observe that southern locations usually have two separate spore seasons (late spring and autumn) while northern locations tend to have one single season detected during summer (Damialis and Gioulekas, 2007; Infante et al., 1999; Marchesi, 2019; Maya-Manzano et al., 2012). Two spore seasons within the same year have been also detected in the north of Africa (Filali Ben Sidel et al., 2015). Some authors have suggested that this heterogeneity in the seasonality of *Alternaria* spore detection is related to the temperature which has been proven to be one of the main factors determining the *Alternaria* spore production (Aira et al., 2013; Maya-Manzano et al., 2012). It has been proposed that the high temperature during mid-summer in the southernmost locations is one of the causes why the MSS have a different temporality than in central Europe. However, there are other environmental factors, such as the water and nutrient availability that have an important role in determining the spore production and release of these saprophytic fungus (Damialis et al., 2015; Grinn-Gofroń et al., 2019; Skjøth et al., 2016).

The seasonal pattern of the *Alternaria* spore season is very relevant to the allergic population, since it could suppose the extension of the exposure to this aeroallergen to periods in which people usually do not take antihistaminic medication, i.e. late summer or autumn (Picornell et al., 2019; Rojo et al., 2016). However, the factors that determine the *Alternaria* seasonal pattern based on one or two seasons remain unclear, and the forecast models that were elaborated for *Alternaria* spores were based on predicting the first (or single) season during the spring-summer period. Therefore, very little is known about the environmental conditions that would favour different seasonal patterns during certain years and locations, based on both one or two seasons (Skjøth et al., 2016).

For all the aforementioned, we hypothesise that the occurrence of one or two *Alternaria* spore seasons within a year may be influenced by site-specific weather conditions. Increasing the knowledge about the environmental conditions that stimulate the production of a second *Alternaria* spore season could be of great interest to reduce the uncertainties about the fungal exposure and the negative impacts that these spores have on public health. Spain is an optimal area to contrast our hypothesis, since it has wide environmental gradients where the factors related to one or two *Alternaria* spore season display can be analysed (Aira et al., 2013; Fernández-Rodríguez et al., 2015; Grinn-Gofroń et al., 2019; Skjøth et al., 2016). Therefore, the main aim of this study was to identify the environmental factors (weather conditions) that determine the number of *Alternaria* spore seasons within a natural year at different sampling locations in Spain.

2. Material and methods

2.1. Spore data

The daily airborne *Alternaria* spore concentrations of 18 sampling locations in Spain were used in this study (Fig. 1). The sampling locations covered a wide latitudinal, altitudinal and bioclimate ranges of the Iberian Peninsula: 36.67°N–43.13°N in latitude; 58–1073 m a.s.l. in altitude; 12.3–19.7 °C average annual temperature and 290.0–1559.4 mm average annual precipitation during the studied period (Table 1). Except for Santesteban and Pamplona, with a temperate macrobioclimate, all the sampling stations have a Mediterranean macrobioclimate (dry period of at least 2 months during summer) (Fig. 1) (Rivas-Martínez et al., 2017). The sampling years varied for the different locations and covered the period 1997–2020 (Table 1, supplementary Fig. S1).

The aerobiological samples were obtained by means of Hirst-type volumetric pollen traps (Hirst, 1952), one per sampling location. The air flow was adjusted to 10 l/min, and the samples obtained were mounted according to the recommended methodology of the Spanish Aerobiology Network (REA) and the European Aerobiology Society (EAS) (Galán et al., 2007, 2014). Samples were analysed with the aid of a light microscope at ×400 or/and ×1000 magnification, depending on the sampling location. The number of longitudinal sweeps analysed in each sample ranged from 2 to 4 (Table 1). Technicians of the Spanish Aerobiology Network trained to recognise aerobiological particles identified the *Alternaria* spores in the samples. These technicians were subjected to periodical quality controls (Oteros et al., 2013). Data were expressed as spores/m^3 of air according to the international aerobiological recommendations (Galán et al., 2017). The methodologies used for spore counting were heterogeneous among the sampling locations (Table 1). However, our analysis is independent to methodological biases because only the seasonality of the spore detection was compared between the sampling locations included in this study.

In the uncommon cases in which a few consecutive days with missing data were detected in the *Alternaria* database (0–25.4 days/year depending on the sampling location; 5.6 days/year as median), these gaps were completed by applying the moving mean interpolation method, as it has been proved to be the interpolation method that provides the lowest errors, as average, when estimating missing data (Picornell et al., 2021). Most of these missing data were located in periods in which the *Alternaria* spore concentrations are expected to be low or moderate. Data was managed

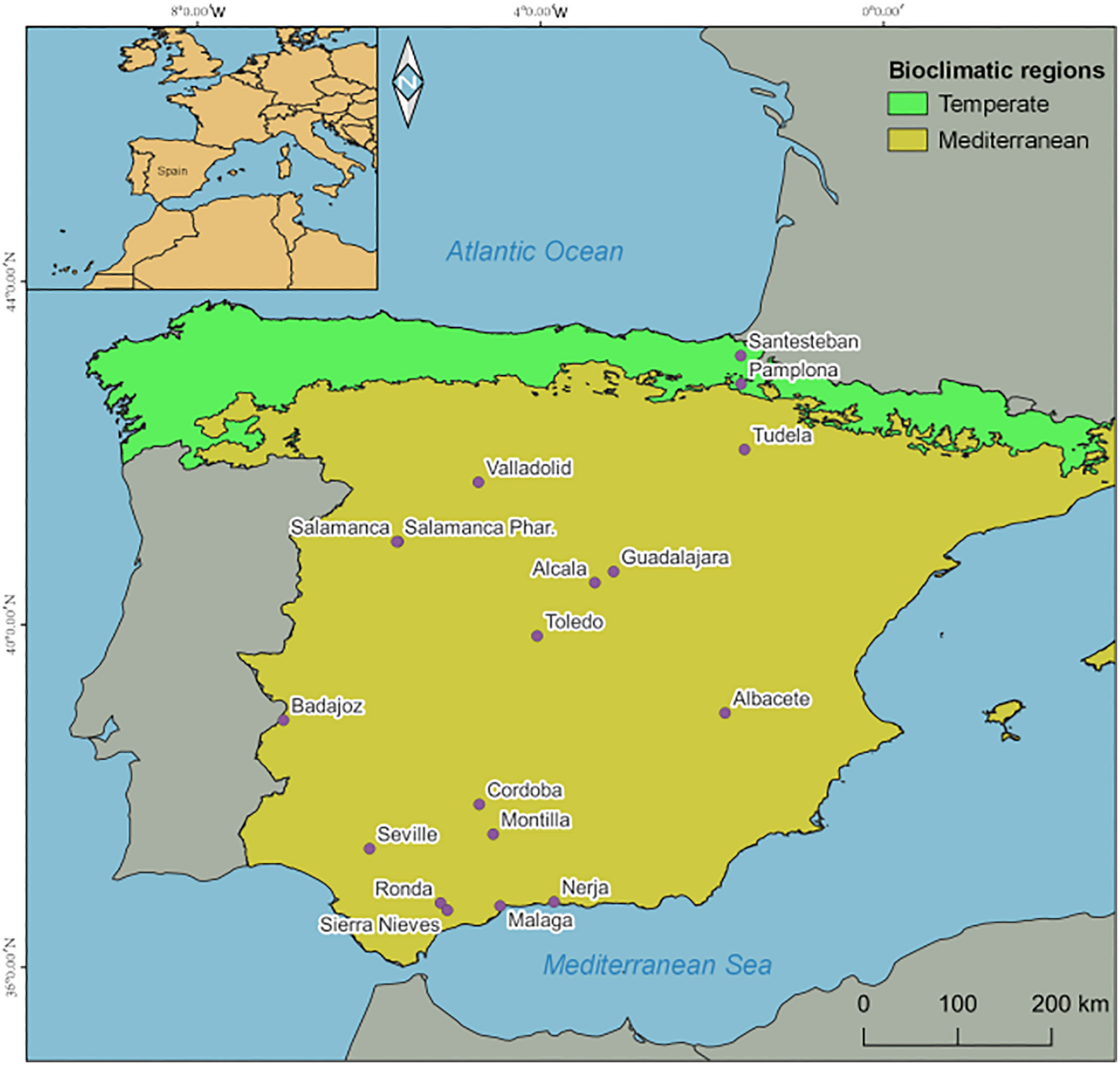

Fig. 1. Map of the sampling locations of *Alternaria* spores used in this study and bioclimatic regions of Spain. Background spatial information obtained from Porto Tapiquén (2015) and Rivas-Martínez et al. (2017). Coordinate system WGS 84. *Alcala*, Alcalá de Henares (Madrid); *Salamanca Phar.*, Salamanca Faculty of Pharmacy; *Sierra Nieves*, Sierra de las Nieves.

Table 1
Sampling periods, counting methodology, location, elevation and weather conditions for each sampling locality. The annual mean temperature and annual total precipitation are the average values for all the studied period in each sampling location.

Locality	Period	Methodology	Latitude	Longitude	Elevation (m)	T (°C)	Prec (mm)
Albacete	2008–2019	4 sweeps ×400	38.96667	−1.85	686	14,9.0	387.3
Alcala	2005, 2006, 2008–2010; 2012, 2014, 2015, 2017–2019	2 sweeps ×400	40.48741551	−3.36831954	588	15.0	361.8
Badajoz	2009–2011	2 sweeps ×400	38.88333	−7.00	190	17.5	562.3
Cordoba	2015–2019	2 sweeps ×400	37.9	−4.716667	138	18.9	445.9
Guadalajara	2008–2016, 2019	4 sweeps ×400	40.61667	−3.15	725	14.1	401.4
Malaga	1997, 2004, 2018, 2019	2 sweeps ×1000/4 sweeps ×400	36.716148	−4.472756	58	18.9	691.9
Montilla	2016–2017	2 sweeps ×400	37.55285	−4.554272	472	17.8	522.9
Nerja	2002–2003	1 sweep ×1000 and 1 sweep ×400	36.76284	−3.845346	170	18.7	525.9
Pamplona	2020	4 sweeps ×400	42.805139	−1.663528	469	12.3	679.1
Ronda	2017–2019	4 sweeps ×400	36.749738	−5.168105	768	16.5	610.7
Salamanca	2014–2018	4 sweeps ×400	40.965364	−5.663928	810	14.1	339.6
Salamanca Phar.	2014–2015	4 sweeps ×400	40.96528	−5.678333	797	14.1	290.0
Santesteban	2020	4 sweeps ×400	43.134721	−1.667207	120	14.2	1559.4
Seville	1997–2003, 2005–2010	2 sweeps ×1000/4 sweeps ×400	37.38306	−5.996389	474	19.7	559.5
Sierra Nieves	2018–2019	4 sweeps ×400	36.665177	−5.089089	1073	14.3	577.3
Toledo	2008–2019	4 sweeps ×400	39.865485	−4.041567	450	16.5	347.3
Tudela	2020	4 sweeps ×400	42.041056	−1.623282	309	14.8	415.9
Valladolid	2005–2006	4 sweeps ×400	41.65889	−4.726667	726	13.1	399.0

Alcala, Alcalá de Henares (Madrid); *Salamanca Phar.*, Salamanca Faculty of Pharmacy; *Sierra Nieves*, Sierra de las Nieves; *T*, anual mean temperature; *Prec.*, annual total precipitation.

with R software and with the aid of the AeRobiology package (R Core Team, 2021; Rojo et al., 2019).

2.2. Detecting one or two Alternaria spore seasons per year

For each sampling location, the average of *Alternaria* concentrations for each day of the year were calculated on the basis of all the available data (e.g. the value for 1st January corresponds to the average of the *Alternaria* daily mean spore concentrations detected the 1st January of all the available years in that sampling location). After that, moving averages of 5 days were calculated with these daily averages. These calculations softened the effect of daily meteorological conditions on *Alternaria* spore concentrations and allowed to obtain a representative curve of the general seasonal behaviour of *Alternaria* spore concentrations for a particular sampling site (i.e. a summary of all the annual spore curves at that location).

However, to objectively determine the number of spore seasons within a year, the monthly *Alternaria* spore integrals were calculated for each sampling location and year. These monthly integrals allowed to detect the periods of the year with the highest *Alternaria* spore detection. Shorter time resolutions may be more affected by the effect of meteorological particularities than monthly resolution. Once these integrals were calculated, the function *findPeaks* implemented in R software was applied to them in order to objectively determine the number of spore seasons within a year (Grinberg, 2019). This function was adjusted to detect those months in each sampling year that had higher average *Alternaria* spore concentrations than the preceding and following 2 months (five-month window).

2.3. Meteorological data

Daily temperature (maximum, minimum and mean temperatures), relative humidity and precipitation data for each sampling location were obtained from the European Climate Assessment and Dataset (ECA&D; www.ecad.eu) (Tank et al., 2002). This database contains information provided by official meteorological stations of the Spanish National Agency of Meteorology (AEMet). For each sampling location, the nearest meteorological station was selected (maximum distance 15.2 km in the case of Badajoz). Given that there were not any meteorological stations near to Sierra de las Nieves sampling location, the meteorological data for this sampling location were obtained from the gridded climate data provided by the E-OBS database from the EU-FP6 project UERRA (www.uerra.eu), the Copernicus Climate Change Service and the data providers of the ECA&D project in its 23.1e version with a spatial resolution of 0.1 degrees (Cornes et al., 2018). The precipitation data for Sierra de las Nieves was obtained from Ronda meteorological station (11.7 km away). Meteorological data of Nerja, Pamplona, Ronda, Santesteban and Tudela sampling locations were obtained directly from AEMet. The meteorological data of Montilla sampling location were obtained from a Campbell-type station located at 200 m away from the Hirst-type sampler.

The Vapour Pressure Deficit (VPD) was calculated from the daily average temperature and daily relative humidity according to the standardized methodology of the Environmental and Water Resources Institute of the American Society of Civil Engineers (Walter et al., 2005).

Seasonal, monthly and annual averages of the meteorological variables were calculated for each year and sampling location. Monthly averages were considered for the period May–November, and seasonal averages were aggregated for the spring March–April-May (MAM), summer June–July-August (JJA) and the autumn September–October-November (SON).

2.4. Determining the optimal weather conditions for the spore production

In order to identify general patterns in the relationship between monthly averages of weather conditions and monthly *Alternaria* spore integrals, LOESS regressions were calculated for each sampling location. This calculation was also elaborated with daily values in the supplementary material. The aim of these regressions was only to depict the general trend and they do not have an intrinsic measurement of the R^2. Therefore, the adjustment of the data to these regressions was calculated by a pseudo-R^2 measurement (R^2loess) proposed by Jacoby (2000). Only monthly temperatures are shown because just this variable showed a clear pattern on the spore concentrations in the atmosphere. The maximum value reached by the fitted LOESS regression was used to define the optimal thermal range for the spore detection.

2.5. Influence of environmental factors on the number of Alternaria spore seasons per year

In order to analyse the most relevant environmental factors that condition the number of *Alternaria* spore seasons within each year, a random forest technique based on classification (for categorical response variable) was applied to model the number of spore seasons. The number of *Alternaria* spore seasons of each sampling year and location was previously established according to the *findPeaks* algorithm explained in section 2.2. Therefore, the categorical responses considered were one or two seasons per year. The predictive variables included in the model were: mean temperature, maximum temperature, minimum temperature, mean relative humidity, mean VPD and accumulated precipitation. These variables were included as seasonal or monthly means of the period March–November as well as annual means. The sampling location was also included as a predictive variable providing intrinsic information with environmental interest (climate, geography, land use, etc.).

The model was trained with the 80% of all the available data, and externally validated with the remaining 20%. These sets of data were randomly selected in order to avoid bias. This procedure was repeated 10 times to average the success rate and the importance of the predictors (cross-validation). The importance of each variable in the model was measured by the mean decrease in the accuracy of the model when this variable was excluded, and by the mean decrease in Gini impurity. The random forest model was created with 500 decision trees by means of the randomForest package implemented in R software (Liaw and Wiener, 2002). In each node of the random forest, 7 variables were considered (mtry = 7). The performance of the random forest in the external validation was measured as the success rate, which was the percentage of correct estimations (Eq. 1).

$$Success\ rate = \frac{N^\circ correct\ estimations}{N^\circ of\ total\ estimations} * 100 \quad (1)$$

Finally, a single regression tree was applied to classify the cases of study (years*stations) in both types of seasonality, with one or two seasons, based on the same predictors that the random forest technique, and using the rpart package (Therneau and Atkinson, 2019). It allows, in an easy way, to study the environmental variables that better distinguish both seasonal patterns for all cases of study. Then, the different locations were represented on the environmental range generated by these environmental key variables.

3. Results

3.1. Alternaria seasonality based on the optimal weather conditions

The highest *Alternaria* spore concentrations were usually detected during the months of May, June, July, September and October in all sampling locations (Fig. 2 and supplementary Fig. S2, S3 and S4; days of the year 121–212 and 244–304). In southern locations such as Badajoz, Cordoba, Malaga, Montilla, Nerja, Ronda and Seville, the highest concentrations

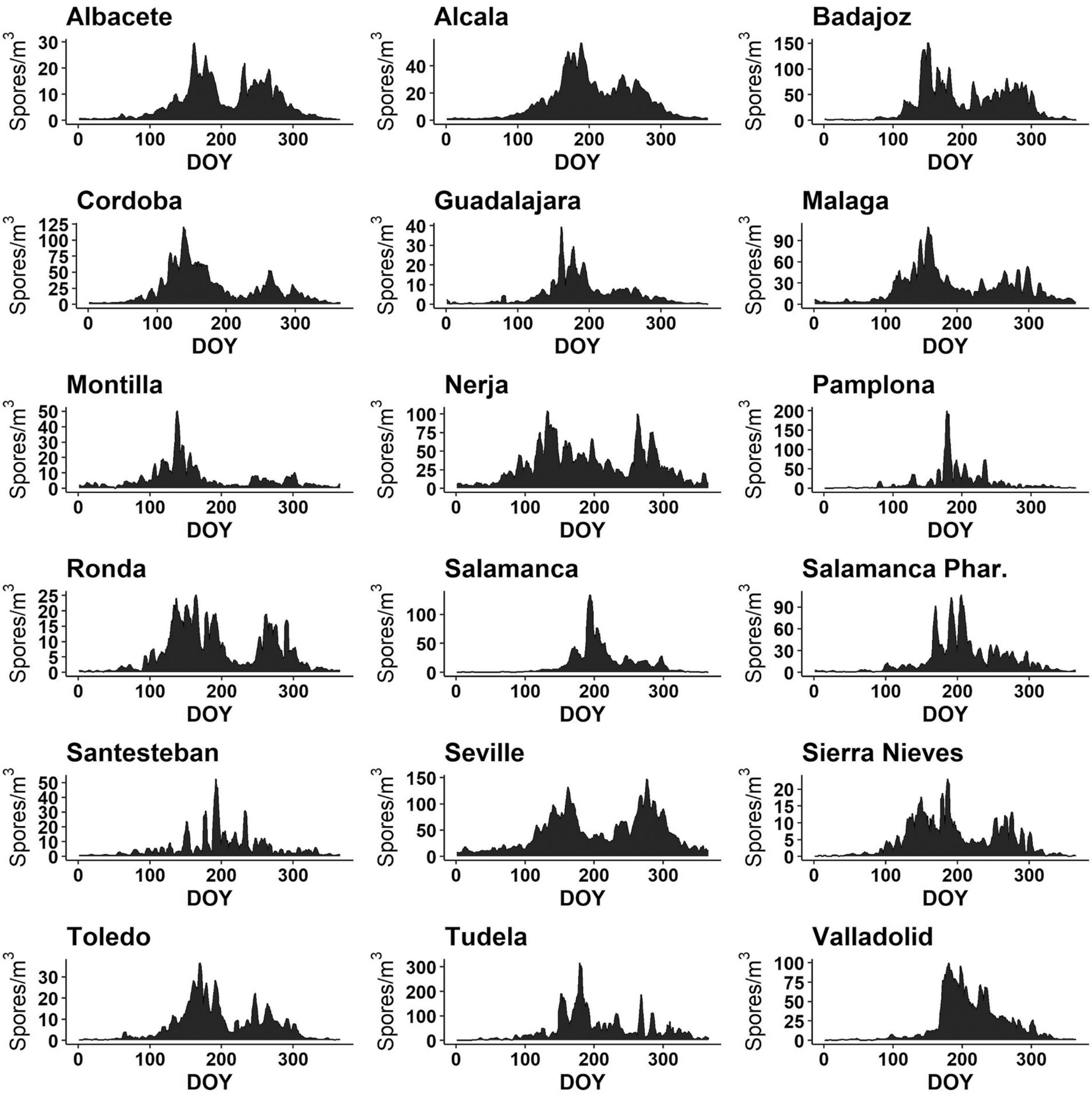

Fig. 2. 5-day moving means of the daily average *Alternaria* spore concentrations for each sampling location during the studied period. *DOY*; day of the year. *Alcala*, Alcalá de Henares (Madrid); *Salamanca Phar.*; Salamanca Faculty of Pharmacy; *Sierra Nieves*, Sierra de las Nieves.

were detected generally earlier than in sampling locations of higher latitudes (e.g., Albacete, Alcalá de Henares, Guadalajara, Pamplona, Salamanca, Santesteban, Toledo, Tudela and Valladolid). In the case of Sierra de las Nieves, despite being in a southern location, it also showed a delay in the detection of its highest *Alternaria* spore concentrations when compared to other near sampling locations. This was observed by comparing the whole studied period for each sampling location (Fig. 2 and supplementary Fig. S2) but also when splitting this period into two halves to avoid the comparison of very distant sampling years (supplementary Fig. S3 and S4).

In general, the overall curve of *Alternaria* spore concentrations in southern locations presented two spore seasons within the same natural year, while the northern locations had a single one (Fig. 2 and supplementary Fig. S2, S3 and S4). For example, locations such as Albacete, Alcala de Henares, Badajoz, Cordoba, Malaga, Nerja, Ronda, Seville, Sierra de las Nieves and Toledo clearly showed two seasons while locations such as Pamplona, Salamanca and Valladolid showed only one. However, at some sampling locations this general pattern had exceptions during certain years, so a year-by-year analysis was necessary to assess the number of peaks of each year (section 3.2).

When analysing the monthly spore integrals and their relationship with the mean temperatures, the highest integrals were detected in the months with temperatures between 18.9 °C and 25.2 °C in most sampling sites (Fig. 3 and supplementary Fig. S5). The integrals tended to decrease when the mean temperatures were below or above this range of temperatures, what suggests that this range was the optimum for *Alternaria* spore dispersion. However, the results showed that when the mean temperature was below 15 °C, the lowest monthly *Alternaria* spore integrals were usually detected in all sampling locations. Similar results were observed when analysing daily data (supplementary Fig. S6).

In accordance with these results, the periods of the years when temperatures were in the range of 18.9–25.2 °C (optimal for high *Alternaria* spore production according to the supplementary Fig. S5) matched with the

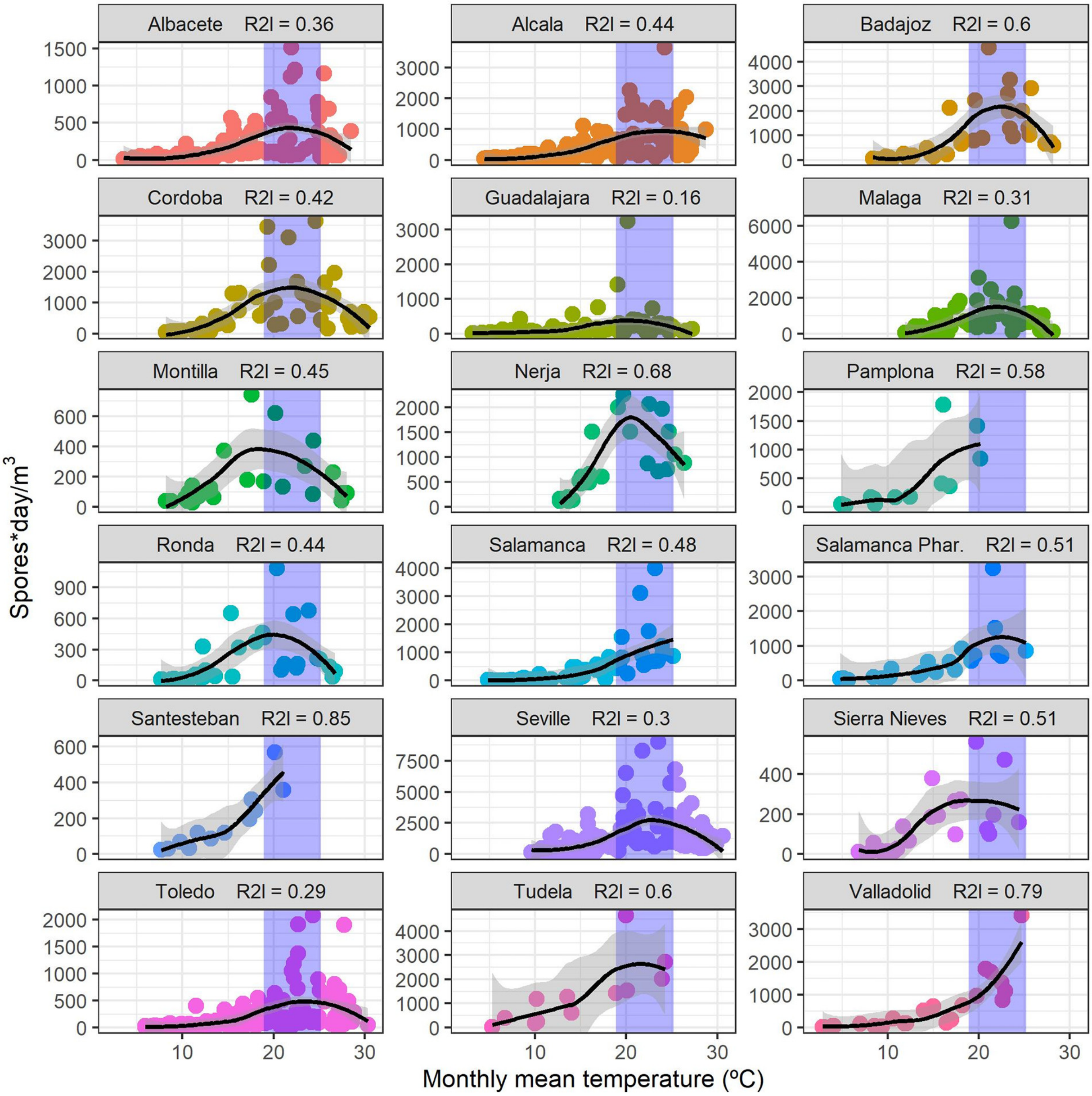

Fig. 3. Relationship between the monthly *Alternaria* spore integral and the monthly mean temperature in all sampled locations during the studied period. The black line represents a LOESS regression, and the grey area represents the confidence interval of the 95%. *R2l*, R^2loess proposed by Jacoby (2000); *Alcala*, Alcalá de Henares (Madrid); *Salamanca Phar.*; Salamanca Faculty of Pharmacy; *Sierra Nieves*, Sierra de las Nieves. The blue area indicates the range between 18.9 and 25.2 °C.

periods showing the highest spore concentrations, and then, this range of temperatures had a great influence on the seasonality observed in the *Alternaria* daily spore concentrations. The Fig. 4 and supplementary Fig. S7 also show two behaviours with respect to seasonal pattern of temperature: the warmer locations exceeded this range during the summer months (e.g. Seville, Cordoba, Malaga, Toledo or Badajoz) and the colder locations usually only reached this range during the summer (e.g. Pamplona, Santesteban, Tudela or Valladolid). In general, mean temperatures in the warmer locations are between 18.9 and 25.2 °C during two periods of the year, namely spring-early summer (May–June) and late summer-autumn (September–October).

3.2. Influence of environmental factors on the number of Alternaria spore seasons

The alternation between one and two *Alternaria* spore seasons at the same sampling location suggested that there were some climate

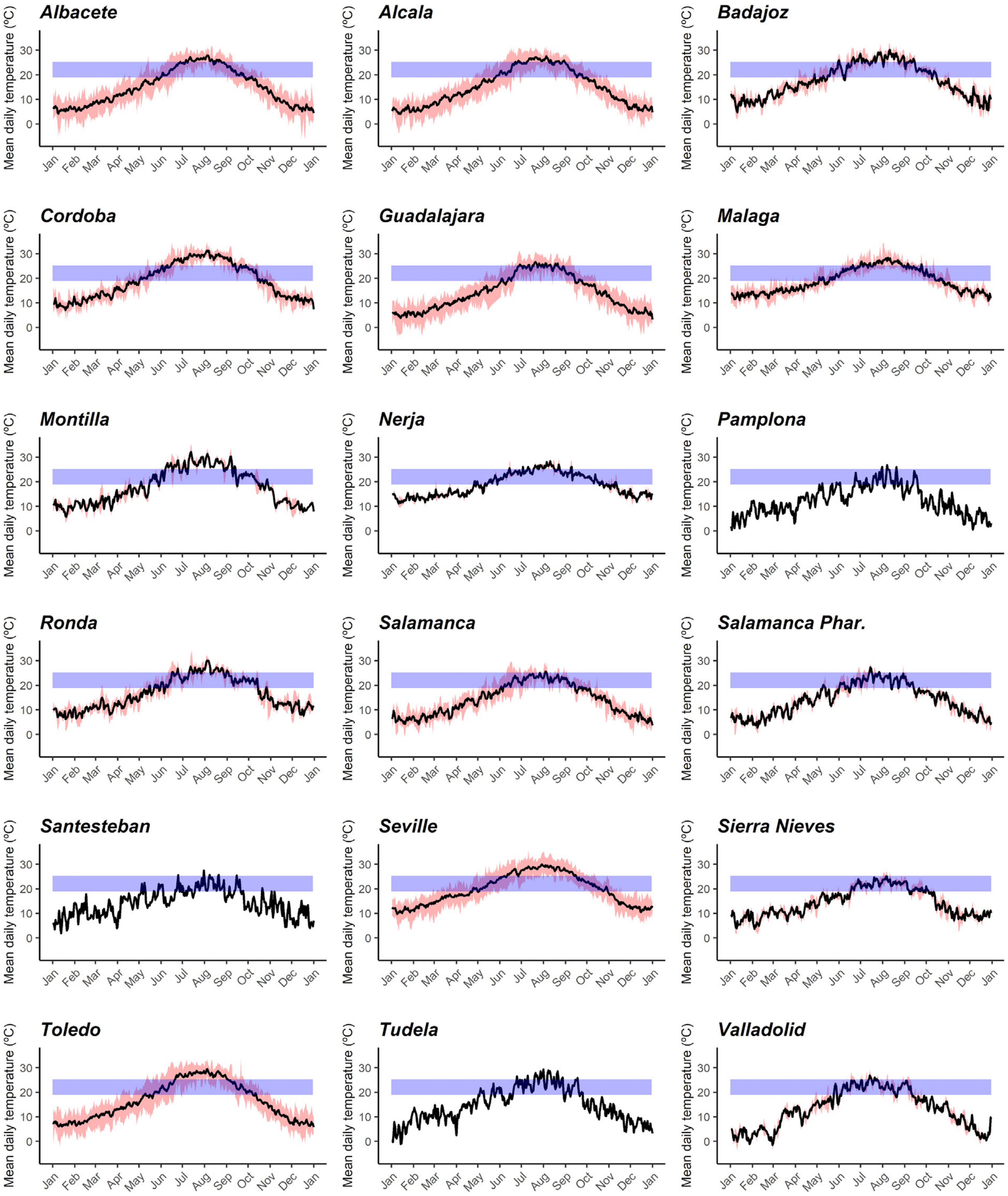

Fig. 4. Seasonal pattern of the temperature for the study sites during the studied period. Average for the mean daily temperature (black line) and amplitude (daily minimum and maximum in red contour). The blue area represents the optimal temperature for the spore production based on Fig. S5 (18.9–25.2 °C). *Alcala*, Alcalá de Henares (Madrid); *Salamanca Phar.*; Salamanca Faculty of Pharmacy; *Sierra Nieves*, Sierra de las Nieves.

factors that changed from one year to other and that were responsible of this alternation. To detect which climate variables were the main contributors that determine if one natural year had one or two *Alternaria* spore seasons, random forest models were generated and the relevance of each variable was measured. The random forest models obtained an average success rate of 73.13% in their external validation, and the most relevant variables for the models were the temperatures and the sampling location (Fig. 5). In particular, the most relevant temperature variables were the annual means of minimum and maximum temperature, and the monthly averages (of mean, maximum or minimum temperature) of April, May, June, August, September and October. The precipitation of June, July and August was also relevant for the model to determine the number of seasons within a year.

3.3. Classification of the spore seasonal patterns based on weather conditions

The minimum and maximum temperature of autumn (TminSON and TmaxSON) and the maximum temperature of summer (TmaxJJA) were the main variables explaining the differences on seasonal patterns between one and two spore seasons. Therefore, both summer and autumn are critical seasons for the *Alternaria* spore production (Fig. 6). Most of the years showed one single spore season when the minimum temperature of autumn was below 11.4 °C and the maximum temperature of summer was below 32.6 °C. On the contrary, higher minimum temperatures during the autumn determined years with two spore seasons. In an intermediate position, when the minimum temperature of autumn was favourable for two spore seasons but the maximum temperature of summer was not (TminSON <11.4 °C and TmaxJJA ≥32.6 °C), the maximum temperature of autumn

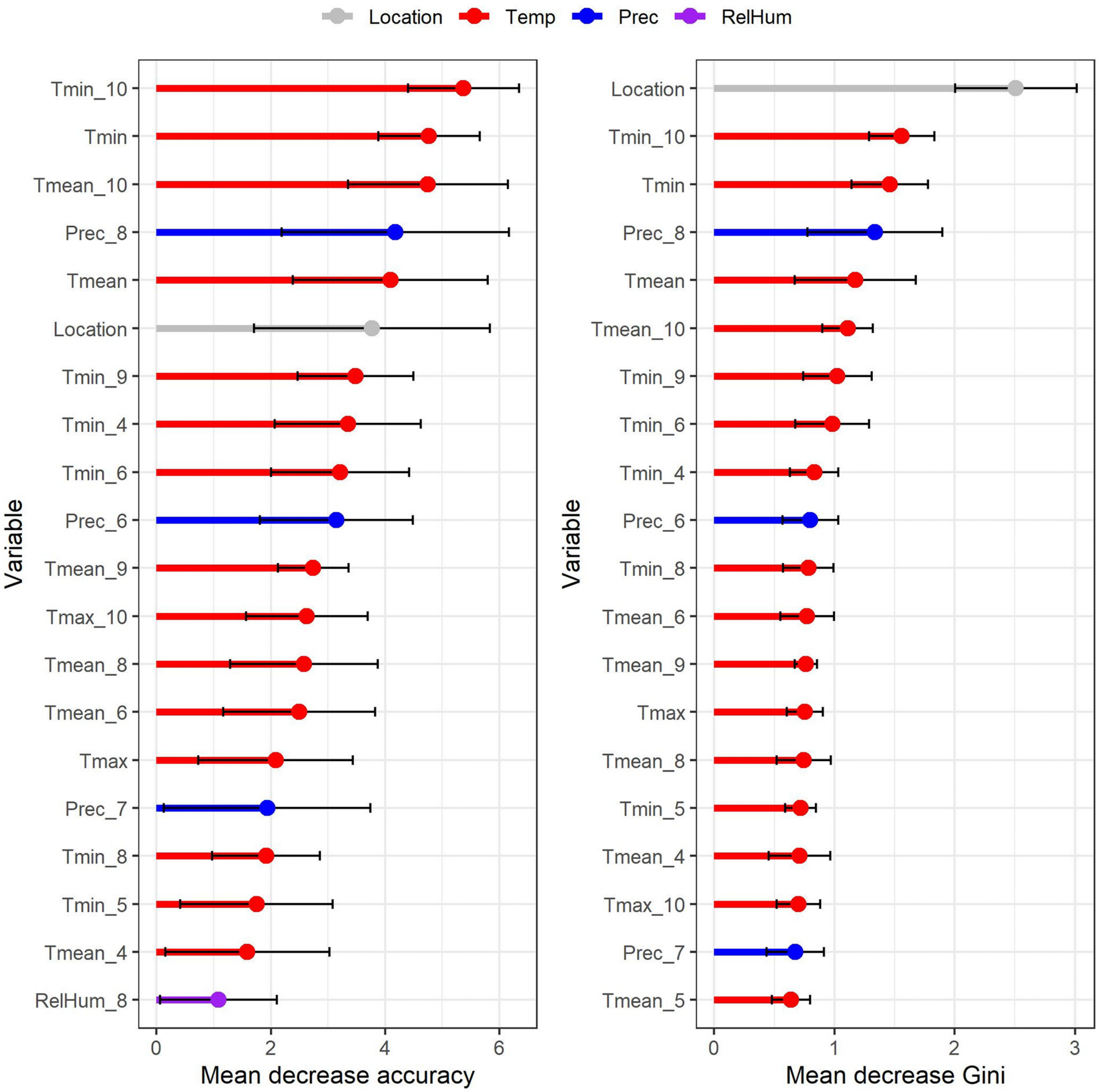

Fig. 5. Mean decrease in accuracy and mean decrease in Gini index when removing the 20 most relevant variables included in the random forest models. The means and the standard deviations were calculated with the results of 10 random forest models (cross-validation). In grey, the sampling location; in red, variables related to temperature; in blue, variables related with precipitation; in purple, variables related with relative humidity. *Tmax*; maximum temperature; *Tmean*, mean temperature; *Tmin*; minimum temperature. Variables without number in their name represent annual means. Variables with the number in their name represent monthly averages and the number indicates the month.

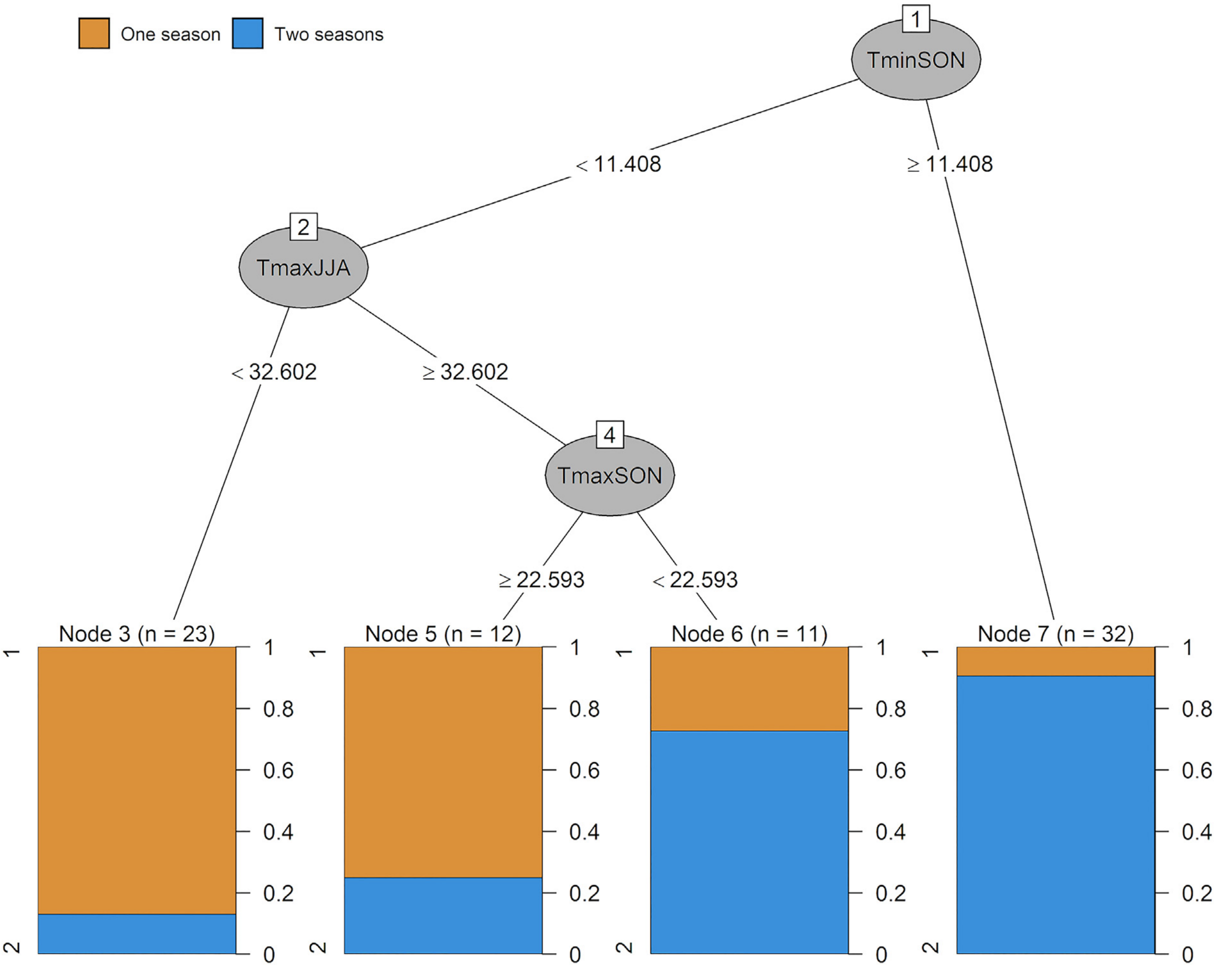

Fig. 6. Results of the regression tree for the classification of the number of *Alternaria* spore seasons (one or two seasons) within the natural years based on weather conditions: minimum temperature of autumn (*TminSON*), maximum temperature of summer (*TmaxJJA*) and maximum temperature of spring (*TmaxSON*).

(TmaxSON) was determinant for the production of one or two spore seasons. Most years with TmaxSON ≥22.6 °C had on single *Alternaria* spore season, while years with lower TmaxSON had two spore seasons.

When representing the sampling locations in the environmental range of minimum and maximum temperature of autumn, and maximum temperature of summer (i.e. the most critical variables for determining the number of *Alternaria* spore seasons; Fig. 6 and supplementary Fig. S8), the warmer locations during these seasons such as Badajoz, Ronda, Montilla, Cordoba, Seville or Malaga clearly showed two seasons during most of the study years. On the other hand, colder locations such as Pamplona, Santesteban, Valladolid, Salamanca, Guadalajara or Tudela showed only a single season during most study years (Fig. 7). Other sampling locations such as Toledo, Albacete or Alcalá de Henares showed an intermediate pattern (i.e. some years with one season and others with two). Some sampling locations with low number of sampling years such as Salamanca Faculty of Pharmacy, Sierra de las Nieves and Nerja showed also an intermediate pattern. Similar results can be observed when analysing individual years (supplementary Fig. S6).

4. Discussion

In general terms, the latitude of the sampling location was related with the phenology of the *Alternaria* spore curve (Fig. 2 and supplementary Fig. S2, S3 and S4): southern locations registered their highest *Alternaria* spore concentrations earlier than the northern locations. In accordance with these results, other studies carried out in central Europe (higher latitudes) reported later *Alternaria* MSS than the locations in the north of the Iberian Peninsula (Grinn-Gofroń et al., 2019; Skjøth et al., 2016). This latitudinal effect was softened by the altitude as it was observed in the case of Sierra de las Nieves sampling location (1073 m a.s.l.), that had a delayed *Alternaria* phenology when compared to other nearby locations. A previous study proved that the differences in altitude had a similar effect than differences in latitude over fungal phenology (Andrew et al., 2018). This can be explained because both latitude and altitude modify the temperature, and this variable has been proven to be one of the most relevant ones in determining the *Alternaria* spore phenology (Andrew et al., 2018; Damialis et al., 2015; Grinn-Gofroń et al., 2019; Skjøth et al., 2016). The seasonality of the sampling locations was studied by comparing data from different periods (supplementary Fig. S1), so it may slightly vary if the same period was compared for all sites. However, these patterns were consistent when comparing data from less distant periods (supplementary Fig. S3 and S4) and, given that most sampling locations had 3 or more sampling years, it is expected that the results represent the general pattern of these locations. In the cases when less than 3 sampling years of data are available, it may be possible that the general pattern of these localities is not accurately represented. Nevertheless, since each year was analysed independently for the rest of this study, the other results are not likely to be affected by this.

Beyond the differences in the phenological behaviour, in this study we analysed differences in the seasonal pattern that have been little studied in literature (Skjøth et al., 2016). These differences were related to the presence of one or two *Alternaria* spore seasons within the same natural year. The presence of a second *Alternaria* season is more common in southern locations (Figs. 2 and 7 and supplementary Fig. S2, S3 and S4) and it was also detected in previous studies at certain southern and middle locations of

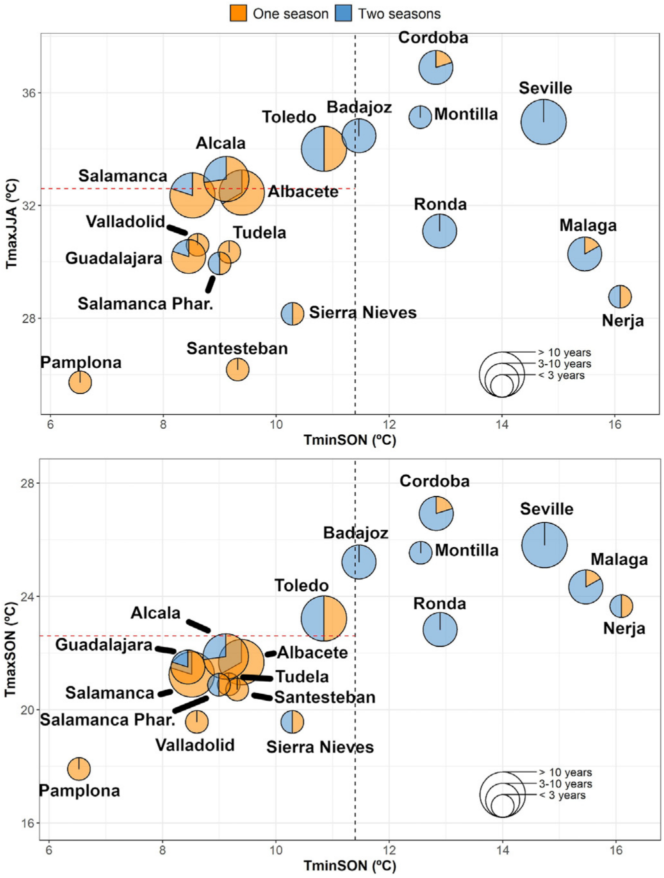

Fig. 7. Distribution of the sampling points on the environmental range based on minimum and maximum temperatures of autumn (*TminSON* and *TmaxSON*) and maximum temperature of summer (*TmaxJJA*). Percentage of the sampling years with one *Alternaria* spore season (orange) and with two *Alternaria* spore seasons in each sampling location. *Alcala*, Alcalá de Henares (Madrid); *Salamanca Phar.*; Salamanca Faculty of Pharmacy; *Sierra Nieves*, Sierra de las Nieves. The size of each chart represents the number of sampling years at that location. Dashed lines indicate the thresholds obtained from the regression tree (Fig. 6).

Spain as well as in other Mediterranean locations at similar latitudes. In these locations, the *Alternaria* seasons were detected during the end of spring and the start of autumn, what matched the seasonality of those detected in this study. However, in locations of Central and Northern Europe, only one single spore season per year is normally detected, as it was observed in the northern locations of Spain (Damialis and Gioulekas, 2007; Grinn-Gofroń et al., 2019; Infante et al., 1999; Marchesi, 2019; Maya-Manzano et al., 2012; Skjøth et al., 2016).

The temperature can explain the detection of one or two *Alternaria* spore seasons within the same natural year. Southern locations normally have warmer temperature conditions than the northern ones. These conditions increase the duration of the favourable period for *Alternaria* spore production and dispersion, but this period is split in two halves (one in May–July and other in September–October) by a brief unfavourable period of excessive warm temperatures in the middle of summer, as observed in the results. During this last period, the temperatures generally exceeded the optimal temperature range for *Alternaria* spore production and dispersion, as it was observed in Fig. 4 and supplementary Fig. S7, and as also suggested previous studies (Damialis et al., 2015; Rodríguez-Rajo et al., 2005).

Therefore, the years that had two main *Alternaria* spore seasons were those that had two periods with mean temperatures in between the optimal range for *Alternaria* spore production and dispersion separated by a period of unfavourable temperatures, exceeding this thermal range. In northern locations, the optimal environmental conditions are generally only reached once per year, during the warmest months of summer, and that is the reason of them having only one *Alternaria* season, and then also showing a shorter spore season. On the opposite, southern locations normally reach these thresholds during late spring or early summer and autumn, and that is why they have two separate *Alternaria* spore seasons (Fig. 4 and supplementary Fig. S7).

The high relevance of the temperature in determining the number of *Alternaria* seasons was also supported by the results of the random forest models and the regression tree (Figs. 5 and 6). The most relevant variables for determining the number of *Alternaria* seasons within the same year were the temperatures of the periods where the highest *Alternaria* spore concentrations were detected (spring-summer and autumn temperatures).

The precipitation detected during the summer months also showed influence in the number of seasons. It may be explained because these fungi require certain water availability together with mild temperature to detonate the spore production. Unfavourable periods marked by very high minimum temperatures and dry conditions provoke the interruption in the process of spore emission (Janić Hajnal et al., 2021), i.e. two separated seasons were observed before and after this unfavourable period (summer). In accordance with these results, both temperature and precipitations proved to be relevant variables to forecast the *Alternaria* spore concentrations in previous studies (Apangu et al., 2020; Grinn-Gofroń et al., 2019). The sampling location was also a very relevant variable given that it is an indirect measurement of the land use and vegetation distribution of the nearby area of the sampling device, what can influence the *Alternaria* spore concentrations in the atmosphere (Fernández-Rodríguez et al., 2015; Grinn-Gofroń et al., 2020; Maya-Manzano et al., 2016). Furthermore, the location variable also constitutes an indirect measurement of the latitude, the annual mean temperature and other climate conditions of the area. In this sense, the temperature is also integrated in the sampling location variable. The other variables related to VPD and relative humidity had much lower relevance in the random forest models.

The relevance of the temperatures in the *Alternaria* spore seasonality was again observed in the relationship between the number of seasons and the temperatures of June–November (Fig. 6). If only these three variables are considered, it is possible to separate the years with one and two seasons in most cases. In general terms, it is necessary that the minimum temperatures during the autumn months exceed 11.4 °C in order to detonate the autumn *Alternaria* season. If the temperatures are too high during summer, these months are unfavourable for spore production and dispersion, and thus, the *Alternaria* spore season is split in two separated seasons. In accordance with the results of Fig. 3, the months with temperatures under approximately 15 °C are usually those with the lowest *Alternaria* spore integrals since this temperature is not enough to stimulate the fungal growth and spore production (Damialis et al., 2015; Janić Hajnal et al., 2021). Additionally, if the maximum temperatures of autumn are too high ($\text{TmaxSON} \geq 22.6$ °C), this period would become less favourable for the *Alternaria* spore production and dispersion, and their concentrations in the atmosphere are likely to be reduced. Therefore, under these circumstances, the autumn *Alternaria* spore season reduces its intensity or even disappears.

In certain sampling locations such as Cordoba, Guadalajara, Salamanca or Malaga, there were some exceptional years when the general pattern of having one or two *Alternaria* seasons per year of that same location was not followed (Fig. 7). In general, these years were characterised by some temperature anomalies in comparison to the other years that followed the general pattern. In warmer locations, when the temperature conditions of certain year were too cold during one of the two favourable periods for *Alternaria* spore production, only one *Alternaria* season was produced during that year (i.e. only a single favourable period was observed). In other cases, the summers were not warm enough to interrupt the favourable period and the optimal conditions for fungal growth and reproduction were more constant, what allowed the development of a single and long *Alternaria* season. In colder locations, warm conditions during certain years could lead to have two separate spore season in locations that usually have just one. In Albacete, Alcala de Henares and Toledo, these alternations of cold and warm years were more usual and thus, the frequencies of years with one or two spore seasons were similar.

In the case of locations with low number of sampling years that showed an intermediate pattern (e.g. Salamanca Faculty of Pharmacy, Sierra de las Nieves or Nerja), the low number of data could induce a bottle-neck effect, and thus, it is not possible to precisely determine their general pattern. This is certainly a limitation of this study.

With an accuracy rate of 73.13%, the random forest obtained could be useful for determining if one year will have one or two *Alternaria* spore seasons. These forecasts would be of great interest for the allergic population in order to reduce their symptoms by taking preventive measures reducing the uncertainty about the seasonality of fungal exposure (Kustrzeba-Wójcicka et al., 2014; Olsen et al., 2020). This model could be improved in future studies by considering also other variables that notably affect the *Alternaria* airborne spore concentrations, such as the nutrient availability (e.g. including land use) or meso- and macro-scale atmospheric events.

5. Conclusions

The temperature is the most relevant variable for the *Alternaria* spore dispersion. It influences both the *Alternaria* spore integrals and its seasonality. The highest *Alternaria* spore concentrations were detected in the months with mean temperatures between 18.9 and 25.2 °C. This optimal range of temperature determines the seasonal pattern of the *Alternaria* main spore season. Also, the water availability is a main factor that determines *Alternaria* spore production. Thus, warmer sampling locations generally have a larger period of *Alternaria* spore detection than the colder sites. If the summer is extremely warm (maximum mean temperatures over approximately 32.6 °C), the favourable period for *Alternaria* spore production and dispersion is split into two separate ones: one in May–July and other in September–October.

Therefore, the appearance of two seasons within a same natural year is due to the interruption of the spore production during summer unfavourable conditions in the warmest areas. The information obtained from these results can be used to alert about the periods of high exposure of allergic population to this bioaerosol. But most importantly, these results allow to extent the knowledge about the environmental conditions influencing the seasonal patterns of airborne *Alternaria* spore, as well as to better understand the periods with high fungal exposure independently of the considered location.

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CRediT authorship contribution statement

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Methodology: A.P and J.R.
Software, validation and formal analysis: A.P and J.R.
Investigation and data curation: all authors.
Writing original draft: A.P. and J.R.
Reviewing and editing the manuscript: all authors.
Visualization: A.P and J.R.
Supervision: A.P., J.R., M.M.T. and M.R.
Project administration: A.P., J.R., M.M.T. and M.R.
Funding acquisition and resources: M.M.T., R.P-B, M. G-B, P·C-M, Z.F., J. M-G, E. S-R, J. SS, I. D., C.G., H. G-M, P.A., S.F., M.G-A, E.R., A.P.Z, A.A. and M.R.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.153596.

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