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Identifying key environmental factors to model Alt a 1 airborne allergen presence and variation

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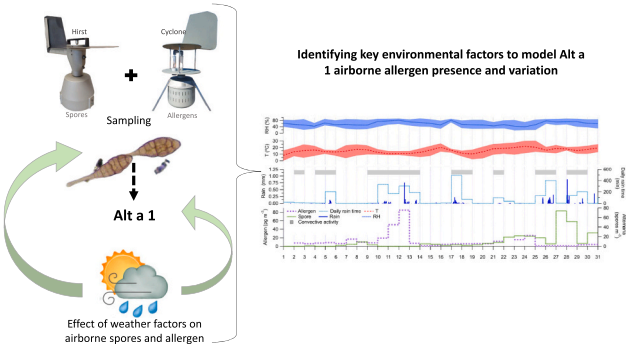
HIGHLIGHTS

- Alt a 1 is present in the air despite low *Alternaria* concentrations.
- Higher monthly temperatures lead to more Alt a 1 days except for the warmest month.
- Quadratic analysis achieves over 67 % accuracy to predict days with allergen.
- Combining spore counts and allergen studies is crucial for allergy risk forecasting.

GRAPHICAL ABSTRACT



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ABSTRACT

Fungal spores, commonly found in the atmosphere, can trigger important respiratory disorders. The glycoprotein Alt a 1 is the major allergen present in conidia of the genus *Alternaria* and has a high clinical relevance for people sensitized to fungi. Exposure to this allergen has been traditionally assessed by aerobiological spore counts, although this does not always offer an accurate estimate of airborne allergen load. This study aims to pinpoint the key factors that explain the presence and variation of Alt a 1 concentration in the atmosphere in order to establish exposure risk periods and improve forecasting models. *Alternaria* spores were sampled using a Hirst-type volumetric sampler over a five-year period. The allergenic fraction from the bioaerosol was collected using a low-volume cyclone sampler and Alt a 1 quantified by Enzyme-Linked ImmunoSorbent Assay. A cluster analysis was executed in order to group days with similar environmental features and then analyze days with the presence of the allergen in each of them. Subsequently, a quadratic discriminant analysis was performed to evaluate if the selected variables can predict days with high Alt a 1 load. The results indicate that higher temperatures and absolute humidity favor the presence of Alt a 1 in the atmosphere, while time of precipitation is related to days without allergen. Moreover, using the selected parameters, the quadratic discriminant analysis to

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predict days with allergen showed an accuracy rate between 67 % and 85 %. The mismatch between daily airborne concentration of *Alternaria* spores and allergen load can be explained by the greater contribution of medium-to-long distance transport of the allergen from the major emission sources as compared with spores. Results highlight the importance of conducting aeroallergen quantification studies together with spore counts to improve the forecasting models of allergy risk, especially for fungal spores.

1. Introduction

Fungal spores constitute a significant component of primary biological aerosol and due to their size range of 1–100 μm they can be classified from fine to large coarse particulate matter (Mandrioli et al., 1998; Després et al., 2012; Yamamoto et al., 2012; Hassett et al., 2015).

Among the great fungal airborne biodiversity, *Alternaria* Ness. is a widespread and saprophytic genus comprising over 300 species (Woudenberg et al., 2013, 2015; Lawrence et al., 2016). Many of these species are recognized as important phytopathogens that affect various crops (Logrieco et al., 2003; Thomma, 2003; Abuley and Nielsen, 2017; Nowakowska et al., 2019). Moreover, *Alternaria* is known for its abundant production of fungal allergens, with up to 17 characterized allergenic proteins (Kustrzeba-Wójcicka et al., 2014; Gabriel et al., 2016). The major allergen, Alt a 1, specifically reacts with >90 % of IgE serum in patients sensitized to *Alternaria* (Postigo et al., 2011; Rodríguez et al., 2021). Alt a 1 is a glycoprotein with a dimeric β-barrel structure, primarily located in the melanin layer of the cell wall of the mature spores and its biological function remains poorly understood (Twaroch et al., 2012; Gómez-Casado et al., 2014). However, emerging evidence suggests that Alt a 1 is related to the processes of spore germination and plant infection (Gómez-Casado et al., 2014; Garrido-Arandia et al., 2016).

The presence of airborne *Alternaria* spores has been associated with respiratory disorders, namely severe asthma (Zureik et al., 2002; Pulimood et al., 2007; Forkel et al., 2021). However, establishing the dominant sensitization to *Alternaria* has proven challenging due to significant epidemiological variations observed among different countries and due to the fact that *Alternaria* sensitization seems to be a triggering factor in the development of poly-sensitization, most likely because of a broad and complex array of cross-reactive allergens that present homologs in several other allergenic sources (Gabriel et al., 2016). In Europe, the average percentage of sensitization to *Alternaria* is estimated at 9 %, whereas in Spain these percentages range from 0.2 % to 1.9 % (Bousquet et al., 2007a, 2007b; Burbach et al., 2009). Notably, in the region of Castilla y León, where the present study was conducted, the percentage of sensitization to *Alternaria* reaches 61 % among the population allergic to fungi (Armentia et al., 2019).

Several aerobiological studies have been conducted in different areas to assess the environmental exposure to this fungus. These studies have revealed significant differences in spore seasonality and concentrations, even among relatively close areas (Kasprzyk et al., 2015; Grinn-Gofroń et al., 2019; Anees-Hill et al., 2022). Temperature, precipitation, and humidity, are considered the main variables influencing the presence of *Alternaria* spores in the atmosphere, with dispersion favored during warm dry periods (Rodríguez-Rajo et al., 2005; O'Connor et al., 2014; Grinn-Gofroń and Bosiacka, 2015). Two distinct spore concentration patterns have been reported in Europe: areas with a Mediterranean climate typically exhibit two spore seasons in spring and autumn, while, northern and higher altitude locations experience a single well-defined period with peak concentrations occurring between July and August (Aira et al., 2013; Kasprzyk et al., 2015; Grinn-Gofroń et al., 2019; Picornell et al., 2022). However, other factors such as land use and prevailing winds must also be considered due to their significant impact on the airborne spore load variations among territories with similar climatic features (Apangu et al., 2020; Grewling et al., 2022; Rodríguez-Fernández et al., 2023).

Traditionally, the threshold value for respiratory symptoms in sensitized individuals has been set at 100 spore m^{-3} (Graversen, 1979). However, allergic reactions have been observed at lower thresholds (Rapiejko et al., 2004; Feo Brito et al., 2012). This is largely attributed to the presence of free allergenic particles in the atmosphere, which are released from airborne spores. This release process is complex, involving interactions among various physical and meteorological factors that influence pollen and airborne spores differently (Rodríguez-Rajo et al., 2011; Wang et al., 2012; Buters et al., 2015; Plaza et al., 2016). Generally, airborne allergen concentrations positively correlate with temperature and negatively correlate with precipitation, while humidity determines less univocal patterns (Vara et al., 2016; Aloisi et al., 2018; Grewling et al., 2020). In addition, thunderstorm days have been associated with an increase of atmospheric allergen load (Idrose et al., 2020). However, the specific key variables and their optimal ranges for allergen release remain largely unknown, especially concerning fungal allergens due to the lack of long-term studies.

In order to fill this gap, this study presents, to the best of our knowledge, the longest sampling period of atmospheric Alt a 1 concentration to date. The marked location of the major emission sources of *Alternaria* spores, and the annual variation of airborne conidia concentration (Rodríguez-Fernández et al., 2023) make León an appropriate territory to investigate the complex relationship between atmospheric factors, Alt a 1 release and the presence of spores.

Therefore, the primary objective of this study is to identify the key factors influencing the presence and fluctuations of this aeroallergen. Furthermore, the study aims to categorize risk days based on these variables in order to improve predictions of Alt a 1 environmental exposure, particularly for individuals sensitive to *Alternaria*.

2. Materials and methods

2.1. Study area

The study was conducted in the city of León (Fig. 1), located in the northwest of the Iberian Peninsula (42°36′ N, 05°35′ W and 819 m a.s.l). This area is characterized by a warm-summer Mediterranean climate (ITACYL and AEMET, 2013; Beck et al., 2018), being the mean temperature of the warmest quarter 19 °C and 4 °C the average temperature of the coldest quarter (Rodríguez-Fernández et al., 2022). The average annual precipitation is 544 mm, being April and May the rainiest months, while summer is the driest period with rainfall frequently in the form of storms (Fernández-Raga et al., 2017). In the 30 km surrounding the city, León shows a remarkable variety of landcover (Fig. 1), which contributes to the presence of a significant amount of primary biological aerosols. In northern areas forest and shrubland are the most abundant vegetation, while grasslands are dominant in areas closer to the city, especially in the westerly direction. Agricultural lands, especially cereal and corn fields, are the dominant land cover in the southern and eastern parts. The land use data were obtained from Castilla y León crops and natural land maps (MCSNCyL), which are available at: http://mcsncyl.itacyl.es/en/inicio.

2.2. Aerobiological sampling

The sampling of *Alternaria* airborne spores and Alt a 1 allergen was carried out from 2016 to 2020. The traps were placed on the terrace of the Veterinary Faculty of the University of León, over 15 m above ground level in a place without obstacles that might hinder the arrival of

air masses from any direction. The traps were placed at least 3 m from each other and from the edge to avoid air turbulence.

Spore concentration was monitored using a Hirst-type (Hirst, 1952) 7-day recording volumetric trap (Lanzoni VPSS 2000) with a suction flow rate of 10 L/min, which was adjusted by means of a handheld Lanzoni flow-meter with air resistance. The installation conditions and operating procedures follow the methodology proposed by CEN legislation EN: 16868:2020 (CEN, 2020). Daily sample analysis was performed by two longitudinal scans at 400× magnification in the effective collecting area, being the area analyzed equated to 8 %, which is more than the 5 % recommended for counting fungal spores (Galán et al., 2021). The spore concentration was expressed as spores m^{-3}, following the terminology recommended by Galán et al. (2017).

The Alt a 1 allergen was sampled with a low-volume Cyclone Burkard sampler (Burkard Manufacturing Co Ltd., Hertfordshire, UK) with a suction flow rate of 16.5 L/min. This trap consists of a continued volumetric sampler based on a single reverse-flow miniature cyclone, having an efficiency of 99 % for particle sizes up to 1.06 μm and 93 % for particle sizes ranging from 0.75 to 0.82 μm (Emberlin, 1995). The air samples were collected every 24 h dry directly in a 1.5-mL Eppendorf vial, which was changed at 1000 UTC every day during the study period and stored at −20 °C.

2.3. Extraction and quantification of Alt a 1

The air samples chosen for Alt a 1 allergen detection were selected based on specific criteria. Firstly, the selection was based on the lowest value of the annual maximum concentration (70 spores m^{-3}) observed during the analyzed years. This value served as a threshold for selecting relevant samples. In addition to the days with concentrations equal to or exceeding 70 spores m^{-3}, the 10 days preceding and following these days were also included for analysis. Moreover, the time span between two relatively close selected periods was also analyzed to capture any potential variation in allergen levels. Finally, if the start or end of the selected period exceeded two weeks within a given month, the entire month containing those days was analyzed, in order to ensure a comprehensive understanding of Alt a 1 allergen levels during prolonged periods of increased concentrations. The determination of Alt a 1 allergen in air samples followed established methodologies with certain modifications (Takahashi et al., 2001; Moreno-Grau et al., 2006; Fernández-González et al., 2010, 2019). Specifically, sonication cycles during sample extraction were introduced as proposed by Aloisi et al. (2019) to enhance extraction efficiency for samples with low pollen/spore concentrations (Rodríguez-Fernández et al., 2023).

Alt a 1 quantification was performed using a modified double sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) method based on Asturias et al. (2003). The monoclonal antibody anti-Alt a 1 (Mab1D6) was used for coating, while the biotin-labeled monoclonal antibody anti-Alt a 1 was used for detection (ROXALL, Zamudio, Spain). Recombinant Alt a 1 was used to create a logistic curve used to determine the concentration of Alt a 1 allergen in the air samples (pg mL^{-1}). The data were subsequently converted to pg m^{-3} according to the volume sampled by the cyclone sampler (Section 2.2). A detailed description of the extraction and quantification process is provided in Supplementary material.

2.4. Environmental data

Hourly weather data from 2016 to 2020 (temperature, relative humidity, absolute humidity, insolation and wind speed/direction) was provided by the National Agency for Meteorology (AEMET), whose nearest station is located at 6 km from the sampling site. Moreover, this agency also provided hourly radar images of Echo Top, which were visually analyzed day to day in order to identify updraft strength. This variable is defined as the maximum height at which the value of a given reflectivity (12 dBZ) is reached. The inclusion of this variable in the study is based on analyzing the potential influence that updrafts may have on the resuspension of spores and/or the allergen, thus explaining variation in their airborne concentrations. In addition, climatic data were obtained from an open access database (www.aemet.es).

Due to the strong local character of rainfall events, the precipitation parameters were measured at the same site as the aerobiological monitoring stations (Section 2.2) by a disdrometer (Blanco-Alegre et al., 2021). The variables obtained from the disdrometer were accumulated

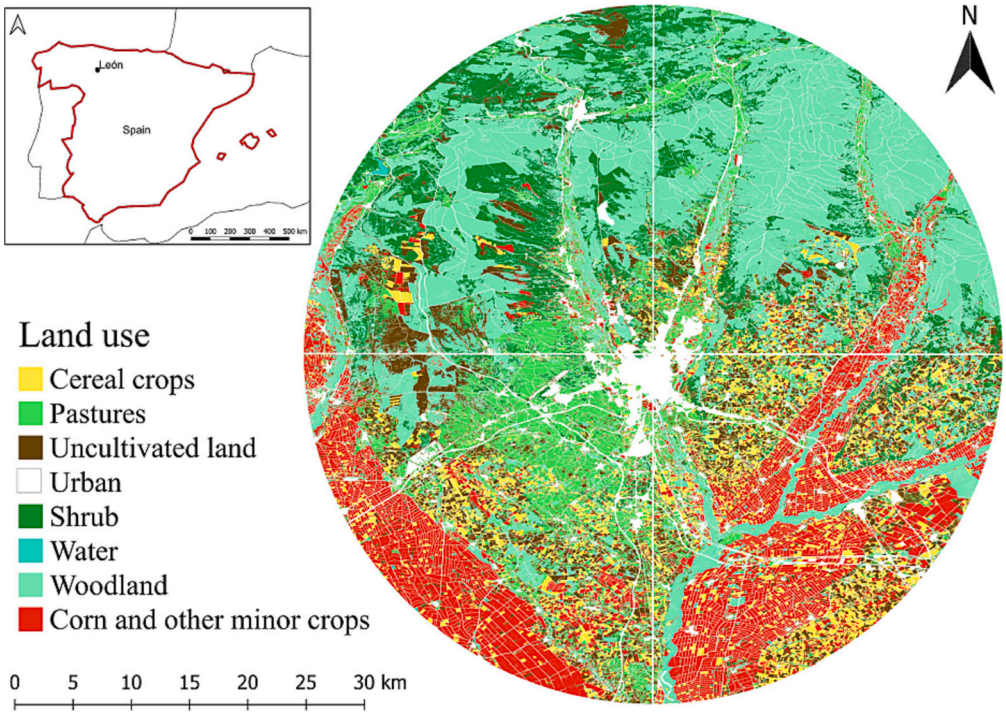

Fig. 1. Geographical location of the city of León on the Iberian Peninsula and land use map within a 30 km radius around the monitoring station.

precipitation, rain intensity and rain duration. Finally, Hourly PM10 concentration was obtained from the air quality network of Castilla y León. These data were analyzed to observe potential variations in their concentrations on days with allergen, which might be associated with the presence of subspore fragments.

The mean concentration of each variable was calculated as a function of the allergen sampling period (24-h). All variables used in the study are summarized in Table 1.

2.5. Statistical analysis

The Alt a 1 data were processed in two steps: i) all days that presented detectable allergen were classified as events, irrespective of the concentration value obtained; ii) days in which the allergen was detectable were divided in three classes by two cut points (tertile 33.3 % and 66.6 %): "low" days (concentration < 1.5 pg m^{-3}), "medium" days (concentration between 1.5 and 4.7 pg m^{-3}) and "high" days (concentration > 4.7 pg m^{-3}).

The non-parametric Spearman rank correlation with a significance level $p < 0.05$ was applied to analyze the relation between variables and allergen presence/concentration. Furthermore, non-hierarchical k-means cluster analysis was performed in order to detect days with similar environmental features (meteorological parameters and spore concentration) that group the allergen events. To carry out this method, the dataset was divided in two, grouping the analyzed months by their meteorological characteristics. On the one hand, the warmer and lower precipitation months (June, July, August and September) were grouped and, on the other, October and November, which present lower temperatures and more precipitation. May was left out of this analysis due to its distinct daily weather features, which could not be meteorologically compared with other days belonging to the analyzed months. The number of optimal clusters was calculated by Ebowl and Silouette methods (Ketchen and Shook, 1996; Rodriguez et al., 2019). Data were managed using different R-packages, as "Factoextra", "Corrplot" and "Vegan" (Kassambara and Mundt, 2016; Wei and Simko, 2017; Oksanen et al., 2020) in R statistical software (http://www.r-project.org/).

Subsequently, a Quadratic Discriminant Analysis (QDA) was individually employed for each cluster, a method to build predictive models using predictor variables (Wang et al., 2018; Blanco-Alegre et al., 2022). This analysis aimed to assess whether the aforementioned parameters can effectively predict the occurrence of days with detectable airborne Alt a 1 concentration. The predicted values obtained from QDA were compared with data observation in each cluster. Then, an error index (Hyvönen et al., 2005) was calculated to check the results (see Supplementary material Eq. 1 & Table S1.). All these calculations were performed using "klaR","MASS" and "biotools" R-packages (da Silva, 2021; Ripley et al., 2023; Roever et al., 2023).

Conditional bivariate probability function (CBPF) was applied in order to study the possible relationship between wind parameters and the Alt a 1 concentration. This method was selected since it allows intervals of concentration (Uria-Tellaetxe and Carslaw, 2014). These intervals were defined by the previously established concentration classes. This analysis was made using "openair" R-package (Carslaw and Ropkins, 2012).

Table 1
Definitions and abbreviations of the variables used in the study. Daily refers to a time period of 24-h.

Variable	Code	Description
Absolute humidity	AbsH	Daily mean absolute humidity (g m^{-3})
Allergen	Alerg	Daily Alt a 1 airborne concentration (pg m^{-3})
Updraft strength	Convec	Detection of Echo top values by radar images in a day
Event	Event	Day with Alt a 1 detected
Insolation	Ins	Daily hours of sunshine (h day^{-1})
Maximum intensity of precipitation	MaxintP	Maximum precipitation intensity recorded in 1 min during the event (mm h^{-1})
Maximum temperature	Tmax	Daily average maximum temperature (°C)
Mean intensity of precipitation	AveintP	Average precipitation intensity during the event (mm h^{-1})
Mean temperature	Tave	Daily average mean temperature (°C)
Minimum temperature	Tmin	Daily average minimum temperature (°C)
Number of dry days	Dryday	Interval of days without precipitation between rainy days (number of days)
Particulate matter<10 μm	PM10	Daily mean PM 10 concentration (μg m^{-3})
Precipitation	P	Daily accumulated precipitation (mm)
Pressure	Press	Daily mean pressure (hPa)
Relative humidity	RH	Daily mean relative humidity (%)
Time of precipitation	TimeP	Duration of precipitation event (min)
Wind direction	WD	Daily mean value of direction (°)
Wind speed	WS	Daily mean values of speed (m s^{-1})

3. Results

3.1. Seasonal variation of Alt a 1 concentration

The lowest annual maximum concentration value registered during the five-year analysis period was 70 spores m^{-3}, which occurred in September 2016 and was set as threshold value. In 2016, although there was a single day with this concentration, the sample period was expanded due to the presence of days with concentration values close to the peak day in July (69 spores m^{-3}). Of the total analyzed days ($n = 501$), 300 of them presented detectable Alt a 1 content. The highest mean allergen concentration (23 pg m^{-3}) was recorded in 2016 and the lowest (2 pg m^{-3}) was observed in 2018 (supplementary Table S2). The highest monthly mean concentration values occurred in September (Table S2), whereas the other months presented heterogeneous values, being in all cases lower than 8 pg m^{-3}.

The number of days with Alt a 1 detected (events) varied depending on the months and years under investigation (Fig. 2). Most of the analyzed months presented a higher number of events, associated to an increase in temperature, especially maximum temperatures. However, a lower number of events was observed with an increase in the temperatures in July. The annual variation of accumulated monthly precipitation did not show a clear relationship with the number of events in the analyzed months (Fig. 2).

The evolution of Alt a 1 concentration exhibits significant fluctuations across the days during the studied periods. Nonetheless, a clear relationship between allergen, spores and the main meteorological variables was not observed (Fig. 3).

3.2. Selection of variables to model Alt a 1 concentration

A Spearman's test was performed to select those variables involved in the presence and variation of airborne Alt a 1. The daily concentration and presence (event) of the allergen had significant positive correlation with MaxintP and RH, while temperature and AbsH presented significant negative correlations (Fig. 4). Moreover, daily Alt a 1 concentration had a very weak but significant correlation with *Alternaria* spore concentration. Instead, spore concentration exhibited a significant positive correlation with temperature, AbsH and PM10, while it showed significant negative correlations with TimeP, MaxintP and Press. The same behavior was observed both by analyzing the years separately as in groups with similar months.

Based on these results, the variables selected to perform the k-means and the quadratic discriminant analysis were: Tmax, Tmin, AbsH, Spore, TimeP and AveintP. Although there was a correlation between Tave and allergen, this variable was excluded because it strongly correlated with other temperature variables. TimeP was selected because of the correlation with spore concentration; MaxintP was discarded because it presented a highly significant correlation with TimeP, so AveintP was chosen instead due to its relationship with MaxintP.

The months analyzed were grouped according to their meteorological characteristics: June, July, August and September (JJAS) on the one

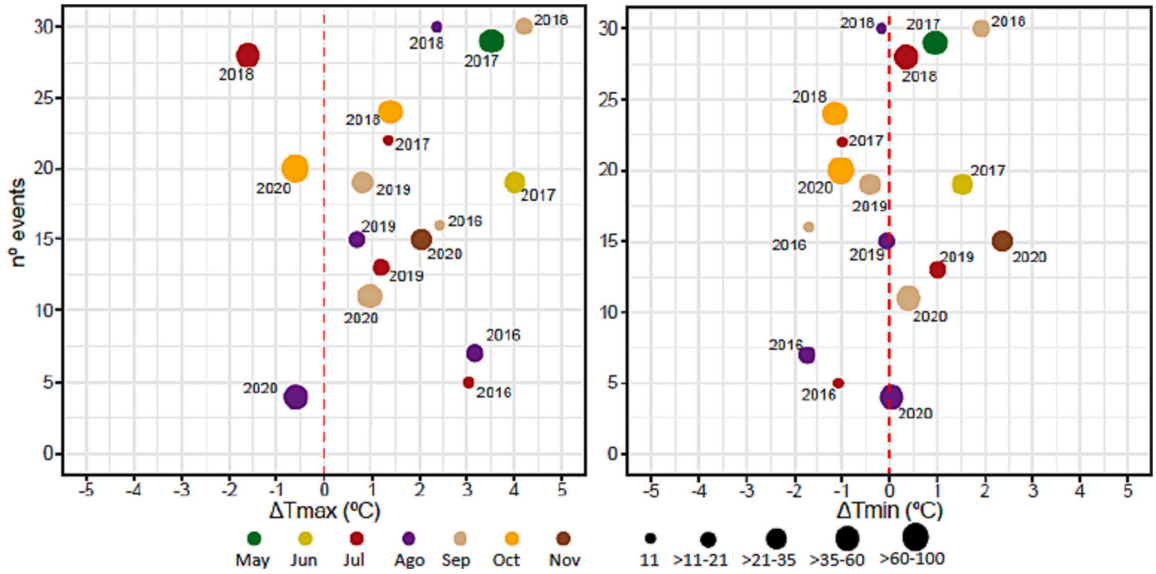

Fig. 2. Number of events (days with Alt a 1 detected) in each analyzed month during the study period and the monthly temperature variation (ΔT °C). ΔT = T′ave – Tcli; being T′ave = maximum or minimum average monthly temperature of the studied year and Tcli = the climatic temperature of the same month. The dot size indicates the monthly precipitation range (mm).

hand, and October and November (ON) on the other. These groups were divided in three clusters, according to the results obtained from Elbow and Silhouette methods (Figs. S1 and S2). The group of warm months (JJAS) includes 397 days. The majority of them were grouped into cluster 1 (Table 2), which includes the highest number of events; most of them presented medium and low concentrations of spores. This cluster was characterized by the highest temperatures and scarce precipitation. The group formed by October and November consisted of 74 days (Table 2); 69 % of them had medium to low concentrations of Alt a 1 (Table 2). In general, mean airborne *Alternaria* spore concentration did not show significant differences among the clusters except for cluster 3 of ON group, which grouped rainy days (Table 2).

3.3. Application of quadratic discriminant analysis

The majority of the events detected in each cluster were well identified by applying the QDA with the same variables as in k-means. The variables Tmin and TimeP were often related to days without allergen (no event), while Tmax and AbsH were associated with event days (see centroids and canonical discriminant coefficients in Tables S3 and S4). However, this pattern changed in cluster 3 of the JJAS group, where Tmin and AveIntP were linked to event days, while AbsH and TimeP were related to no-event days. The error index varied depending on the cluster (Table 3). The greatest error was obtained in cluster 1 of the JJAS group, which had the largest number of days (239), discriminating well 67 % of the cases. In the other clusters, the prediction accuracy rate was above 70 %. The QDA was not applied to cluster 2 of ON group because it only presented 12 days in total.

3.4. Influence of wind on Alt a 1 concentration

Days with low Alt a 1 concentration were associated with wind coming from northeastern directions at a speed range of 2–4 m s^{-1}. On the contrary, days with high Alt a 1 concentration coincided with days characterized by winds blowing from the south, mainly with speeds ranging from 1 to 4 m s^{-1}. Moreover, days with allergen concentration >4.7 pg m^{-3} were also observed when the wind came from the southwest at faster speeds (5–7 m s^{-1}). Days with medium concentrations had lower probability values and occurred at low wind speeds (0–3 m s^{-1}) mainly blowing from the north (Fig. 5).

4. Discussion

Alternaria spores and their major allergen Alt a 1 showed significant differences in their atmospheric occurrence during the study period. It was previously shown that León is one of the European cities with the lowest *Alternaria* airborne spore concentration (De Linares et al., 2010; Kasprzyk et al., 2015; Grinn-Gofroń et al., 2019), likely due to prevailing winds that hinder the arrival of air masses with high spore loads from the major emission sources (Rodríguez-Fernández et al., 2023). However, a large proportion of days had detectable Alt a 1 allergen levels, despite the low airborne spore concentration. Similar results have been observed in other locations for both *Alternaria* spores (Barnes et al., 2000; Feo Brito et al., 2012) and pollen allergens (Fernández-González et al., 2011, 2013; Aloisi et al., 2018). These complex airborne dynamics are due to the differences in release and dispersion mechanisms between these particles (Plaza et al., 2016; Lara et al., 2023).

The Alt a 1 allergen was detected in air samples collected by a cyclone low-volume sampler, in contrast to the previous results published by De Linares et al. (2022). The discrepancy in quantifying/detecting Alt a 1 is likely attributed to variations in the allergen quantification methods, specifically ELISA. The sensitivity of ELISAs can vary significantly depending on the type of ELISA used (competitive, indirect, or sandwich), the antigens being detected, and the antibodies utilized. This difference in sensitivity is more probable than any inefficiency in the sampler, considering that the cyclone sampler has demonstrated greater efficiency than certain high-volume samplers (Plaza et al., 2017; Suanno et al., 2022). Furthermore, the Alt a 1 concentrations were similar to those reported by Grewling et al. (2020) using a high-volume sampler, despite the great differences in spore concentration between the two locations (Kasprzyk et al., 2015). These findings suggest that the use of the ELISA methodology proposed in this paper, implemented with sonication during sample preparation to enhance allergen extraction (Aloisi et al., 2019), can be used to detect Alt a 1 allergen in low-volume samples, even in the case of low airborne spore concentration. However, one of the most significant limitations of using low-volume cyclonic samplers, and consequently of this study, is their inability to separate intact spores from subspore fragments containing allergens. This makes it difficult to determine the major source of airborne Alt a 1.

The mean Alt a 1 concentration varied by up to 10-fold over the study period, with the highest value recorded in 2016, which had the lowest

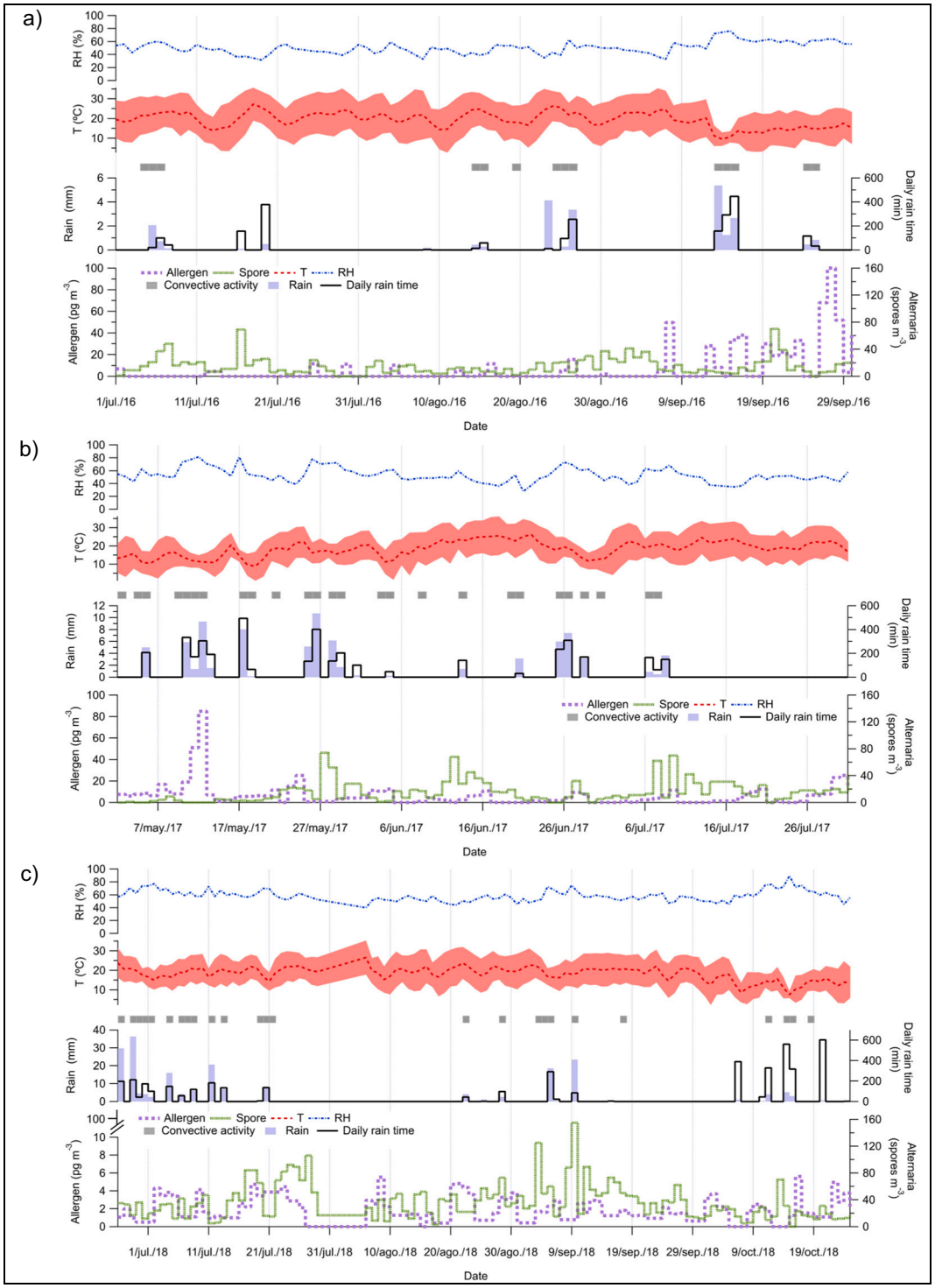

Fig. 3. Daily evolution of Alt a 1 allergen, *Alternaria* spores and meteorological variables during the sampling period in: a) 2016; b) 2017; c) 2018; d) 2019; e) 2020.

spore counts (Rodríguez-Fernández et al., 2023). The low spore concentration in that year can be attributed to the high mean maximum temperature registered in July and August, as high temperatures during the main spore season are a limiting factor for spore production in Mediterranean areas (Damialis et al., 2015; Picornell et al., 2022). These findings coincide with the results from others studies, which have shown that the greatest allergenic potential values are recorded in warmer and drier years with a low spore or pollen concentration (Buters et al., 2010; Fernández-González et al., 2010; Grewling et al., 2019). However, the annual variation of Alt a 1 concentration is complex, and other variables need to be considered. For example, allergen concentration varies among *Alternaria* strains and growing conditions (Ibarrola et al., 2004; Martínez-Quesada et al., 2006). In addition, other genera of the Pleosporaceae family can also contribute to the allergenic airborne load (Gutiérrez-Rodríguez et al., 2011; Teifoori et al., 2019), although no significant concentrations of other fungal spore taxa were observed during the studied period (data not shown).

It is noteworthy that the higher Alt a 1 concentration showed a clear

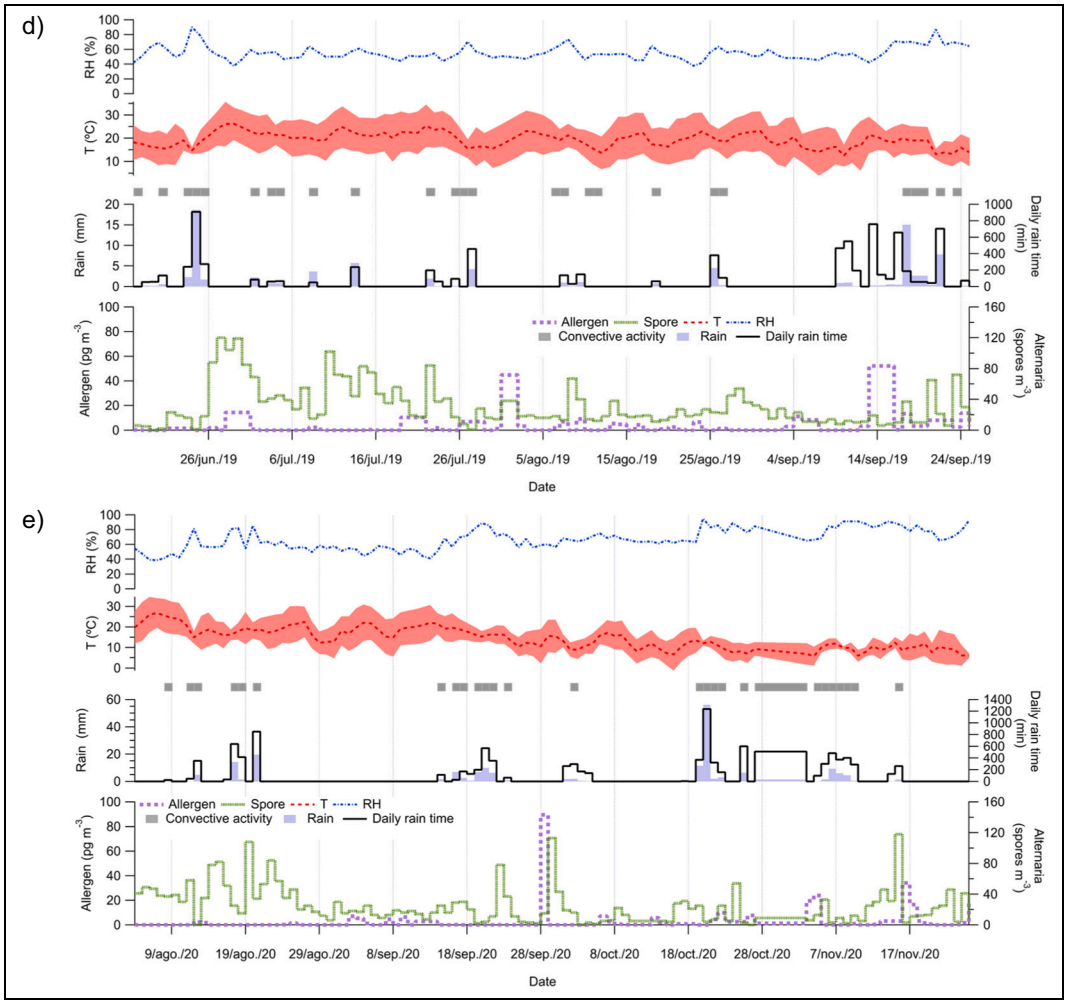

Fig. 3. (*continued*).

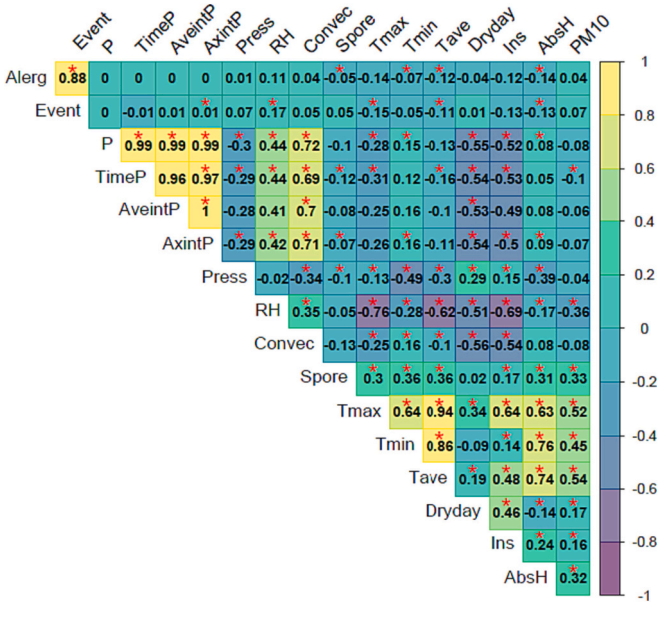

Fig. 4. Spearman correlation matrix coefficients and the significance level $p < 0.05$ (*) for Alt a 1 concentration (Alerg), events, meteorological parameters, *Alternaria* spores and PM10 for the study period.

Table 2
Summary of cluster day characteristics analyzed by k-means for the months June, July, August and September (JJAS) and October and November (ON) during the study period.

	JJAS			ON		
	C1	C2	C3	C1	C2	C3
Total days	239	34	124	41	12	21
Events	131	21	68	31	9	11
Days with high levels	38	6	30	9	1	1
Days with medium levels	50	7	20	14	3	6
Days with low levels	43	8	18	8	5	4
Tmax (°C)	29 ± 3	23 ± 5	24 ± 4	16 ± 4	22 ± 3	15 ± 4
Tmin (°C)	13 ± 2	13 ± 2	8 ± 2	4 ± 2	9 ± 1	8 ± 2
P (mm)	0.3 ± 1.0	9 ± 9	0.1 ± 0.6	0.1 ± 0.1	0	6 ± 12
TimeP (min)	14 ± 39	355 ± 235	14 ± 44	11 ± 37	0	390 ± 246
AveintP(mm min^{-1})	0.3 ± 1.0	4 ± 5	0.1 ± 0.3	0.1 ± 0.1	0	0.8 ± 1.0
AbsH (g m^{-3})	10 ± 1	10 ± 1	8 ± 1	6 ± 1	8 ± 1	8 ± 1
RH (%)	52 ± 8	69 ± 15	55 ± 8	70 ± 11	64 ± 10	77 ± 13
Dry days	200	6	110	37	12	0
Alternaria (spores m^{-3})	34 ± 26	21 ± 27	18 ± 17	15 ± 14	20 ± 13	1

Table 3
Contingence table obtained from discriminant analysis for each cluster for the selected periods: June, July, August and September (JJAS) and October and November (ON).

JJAS												
C1		Pred.		C2		Pred.		C3		Pred.	
		Yes	No			Yes	No			Yes	No
Ob	Yes	107	24	Ob	Yes	16	5	Ob	Yes	62	6
	No	55	53		No	2	11		No	25	31
Error = 33.1 %				Error = 20.6 %				Error = 25 %			

ON							
C1		Pred.		C3		Pred.	
		Yes	No			Yes	No
Ob	Yes	22	9	Ob	Yes	11	0
	No	2	8		No	3	7
Error = 26.8 %				Error = 14.3 %			

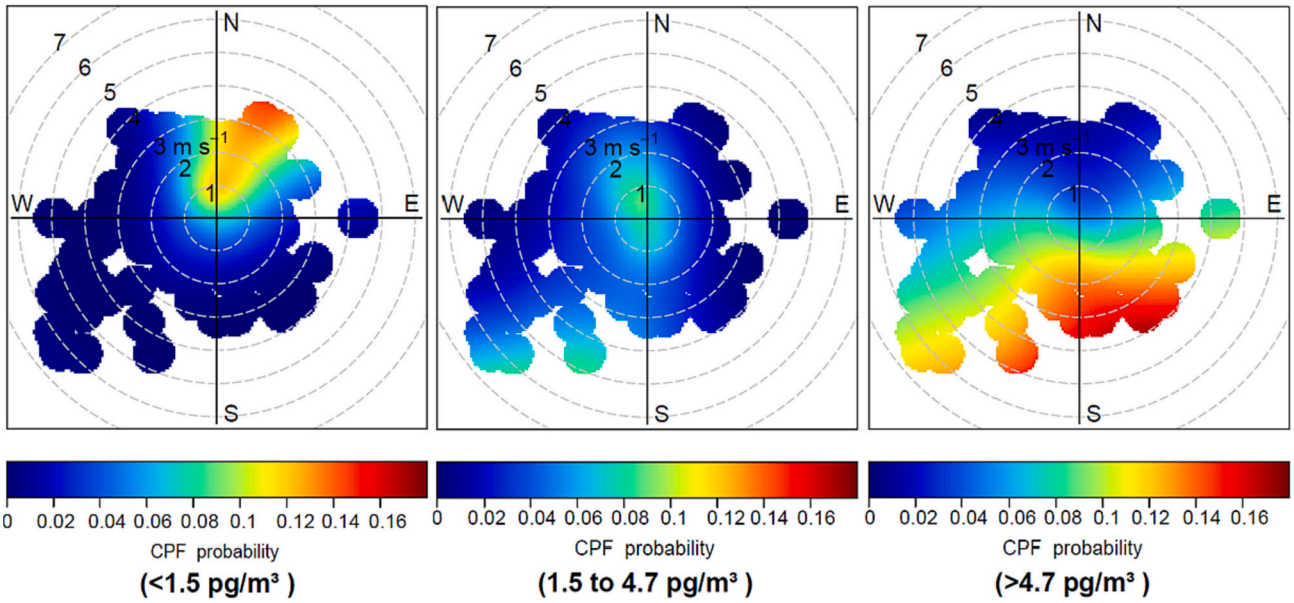

Fig. 5. Conditional bivariate probability function (CBPF) plot for different Alt a 1 concentration levels during the study period as a function of wind speed and direction. Low level (<1.5 pg m^{-3}), medium level (1.5 to 4.7 pg m^{-3}) and high level (>4.7 pg m^{-3}).

pattern of occurrence, unlike days with high spore concentration (Rodríguez-Fernández et al., 2023). The highest mean allergen concentrations occurred in September in most years, likely due to mild temperatures and increased precipitation (Barnes et al., 2000; Feo Brito et al., 2012; De Linares et al., 2022). These weather conditions favor spore germination and thus the release of the Alt a 1 allergen (Hatzipapas et al., 2002; Ibarrola et al., 2004). However, this pattern may be altered in years with unusual meteorological conditions, as is the case of May 2017 in our study. The unusual increase in temperatures while maintaining the average precipitation produced favorable conditions for spore germination and allergen release. In addition, a higher number of allergen days seems to be favored by an increase of mean monthly temperatures, except in July, which is the warmest month in this territory. These monthly variations caused an increase in the maximum temperature range up to 20–29 °C, thus providing optimal conditions for spore germination (Hatzipapas et al., 2002).

The weak correlation values between Alt a 1 allergen and the analyzed variables may be attributed to differences in particle dispersion time from emission sources to the sampler and the varying impact of meteorological factors on airborne spore and allergen concentrations during medium-long distance transport (Lara et al., 2023). This is particularly likely because the major *Alternaria* emission sources around León are located >30 km from the monitoring station (Rodríguez-Fernández et al., 2023). Despite this, no increase in correlations was observed when a time lag of up to 10 days was considered (data not shown).

According to the selected variables, most events occurred on days with scarce rainfall, maximum temperatures around 29 °C, and minimum temperatures around 13 °C. These weather conditions are close to the optimum conditions that favor the dispersion of *Alternaria* spores (Fernández et al., 1998; Rodríguez-Rajo et al., 2005). Moreover, these results concur with the seasonal distribution of symptoms in people sensitized to fungi in Castilla y León (Armentia et al., 2019). However, it is difficult to attribute symptoms exclusively to the presence of Alt a 1, as most patients sensitized to fungi are also allergic to other allergens (Feo Brito et al., 2012; Armentia et al., 2019). It is worth noting that in October and November, although fewer days were analyzed due to low daily *Alternaria* concentration, many of them were characterized by high Alt a 1 content. This evidence might suggest the importance of extending allergen load analysis also to these months in future research.

The QDA correctly classified between 67 % and 85 % of the events, which is similar to the accuracy of others models for predicting spore and pollen concentration (Grinn-Gofroń and Strzelczak, 2008; Astray et al., 2016). Temperature and absolute humidity were the most important variables for predicting the presence of allergen in the atmosphere, while the duration of precipitation was related to the absence of airborne allergen. These results partially agree with the patterns observed in previous studies (Grewling et al., 2019, 2020; De Linares et al., 2022), which support the idea that warm dry periods favor the presence of Alt a 1 in the atmosphere. However, our model also showed the necessity of humid conditions for the occurrence of Alt a 1, since *Alternaria* species germinate at high humidity values (Hatzipapas et al., 2002).

The aforementioned variables explain a significant portion of the variation in airborne allergen concentration. However, allergen concentration also exhibited large fluctuations across different days, which may be attributed to the impact of wind on airborne allergen transport. Days with the highest concentrations were characterized by winds from the south, which also facilitate the transport of spores from the major emission sources to the city (Rodríguez-Fernández et al., 2023). This highlights the close relationship between *Alternaria* spore concentration and Alt a 1 concentration. However, it also highlights differences in their aerobiological transport, since the highest Alt a 1 concentrations are generally linked to higher wind speeds than to *Alternaria* spores in this area (Rodríguez-Fernández et al., 2023). This could be attributed to mechanical rupture of spores by higher wind speeds, as described for certain pollen grains (Caronni et al., 2021; Emmerson et al., 2021), leading to the release of subspore fragments containing allergens and/or free Alt a 1 molecules. This may indicate that medium-to-long distance transport of the subspore fragments or allergen contributes more than spore transport. Indeed, *Alternaria* conidia have an aerodynamic diameter of ~10 μm (McCartney et al., 1993), while allergens or spore fragments are sub-micrometric particles that can be transported over long distances (Plaza et al., 2016).

5. Conclusions

Our findings revealed a weak correlation between the concentrations of Alt a 1 and *Alternaria* spores, possibly due to the effect of weather factors on the release and transport processes of allergens and spores. Hence, it is crucial to measure airborne allergen concentrations in addition to spore concentrations to gain a comprehensive understanding of allergen exposure. On the other hand, temperature, absolute humidity and precipitation, together with wind patterns, can be utilized to predict allergen days, providing valuable insights for allergy risk assessment and recognising the importance of medium to long-distance transport of allergens in determining atmospheric allergen loads.

In the future, incorporating these findings into allergy risk prediction models, particularly for fungal spores, should be considered to improve the accuracy of allergy risk assessments. This would greatly benefit individuals susceptible to *Alternaria* allergies by providing more reliable and timely information to manage their exposure and mitigate potential health risks.

Further research should deepen our understanding of the mechanisms underlying Alt a 1 production, release, and transport. Finally, improving aerobiological sampling should not be neglected, in order to discriminate between entire spores and sub particles bearing allergens.

CRediT authorship contribution statement

Alberto Rodríguez-Fernández: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Iris Aloisi:** Writing – review & editing, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation. **Carlos Blanco-Alegre:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Ana María Vega-Maray:** Writing – review & editing, Validation, Supervision, Resources, Investigation, Data curation. **Rosa María Valencia-Barrera:** Writing – review & editing, Validation, Supervision, Resources, Investigation, Data curation. **Chiara Suanno:** Validation, Resources, Investigation, Data curation. **Ana Isabel Calvo:** Writing – review & editing, Supervision, Resources, Investigation. **Roberto Fraile:** Writing – review & editing, Supervision, Resources, Project administration, Investigation. **Delia Fernández-González:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition.

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.

Data availability

The authors do not have permission to share data.

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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.2024.170597.

References

Abuley, I.K., Nielsen, B.J., 2017. Evaluation of models to control potato early blight (*Alternaria solani*) in Denmark. Crop Prot. 102, 118–128. https://doi.org/10.1016/j.cropro.2017.08.012.

Aira, M.J., Rodríguez-Rajo, F.J., Fernández-González, M., Seijo, C., Elvira-Rendueles, B., Abreu, I., Gutiérrez-Bustillo, M., Pérez-Sánchez, E., Oliveira, M., Recio, M., Tormo, R., Morales, J., 2013. Spatial and temporal distribution of *Alternaria* spores in the Iberian Peninsula atmosphere, and meteorological relationships: 1993–2009. Int. J. Biometeorol. 57 (2), 265–274. https://doi.org/10.1007/s00484-012-0550-x.

Aloisi, I., Del Duca, S., De Nuntiis, P., Vega Maray, A.M., Mandrioli, P., Gutiérrez, P., Fernández-González, D., 2018. Behavior of profilins in the atmosphere and in vitro, and their relationship with the performance of airborne pollen. Atmospheric Environ. 178 (January), 231–241. https://doi.org/10.1016/j.atmosenv.2018.01.051.

Aloisi, I., Del Duca, S., De Nuntiis, P., Mandrioli, P., Fernández-González, D., 2019. Comparison of extraction methods for Poaceae pollen allergens. Aerobiologia 35 (1), 195–200. https://doi.org/10.1007/s10453-018-9538-2.

Anees-Hill, S., Douglas, P., Pashley, C.H., Hansell, A., Marczylo, E.L., 2022. A systematic review of outdoor airborne fungal spore seasonality across Europe and the implications for health. Sci. Total Environ. 818, 151716 https://doi.org/10.1016/j.scitotenv.2021.151716.

Apangu, G.P., Frisk, C.A., Adams-Groom, B., Satchwell, J., Pashley, C.H., Skjøth, C.A., 2020. Air mass trajectories and land cover map reveal cereals and oilseed rape as major local sources of *Alternaria* spores in the midlands. UK. Atmospheric Pollut. Res. 11 (9), 1668–1679. https://doi.org/10.1016/j.apr.2020.06.026.

Armentia, A., Martín-Armentia, S., Moral, A., Montejo, D., Martin-Armentia, B., Sastre, R., Fernández, S., Corell, A., Fernández, D., 2019. Molecular study of

hypersensitivity to spores in adults and children from Castile & Leon. Allergol. Immunopathol. 47 (4), 350–356. https://doi.org/10.1016/j.aller.2018.10.002.
Astray, G., Fernández-González, M., Rodríguez-Rajo, F.J., López, D., Mejuto, J.C., 2016. Airborne Castanea pollen forecasting model for ecological and allergological implementation. Sci. Total Environ. 548–549, 110–121. https://doi.org/10.1016/j.scitotenv.2016.01.035.
Asturias, J.A., Arilla, M.C., Ibarrola, I., Eraso, E., González-Rioja, R., Martínez, A., 2003. A sensitive two-site enzyme-linked immunosorbent assay for measurement of the major *Alternaria alternata* allergen Alt a 1. Ann Allergy Asthma Inmunol. 90, 529–535. https://doi.org/10.1016/S1081-1206(10)61846-7.
Barnes, C., Schreiber, K., Pacheco, F., Landuyt, J., Hu, F., Portnoy, J., 2000. Comparison of outdoor allergenic particles and allergen levels. Ann. Allergy Asthma Immunol. 84 (1), 47–54. https://doi.org/10.1016/S1081-1206(10)62740-8.
Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future köppen-geiger climate classification maps at 1-km resolution. Scientific Data. 5, 1–12. https://doi.org/10.1038/sdata.2018.214.
Blanco-Alegre, C., Castro, A., Calvo, A.I., Oduber, F., Fernández-González, D., Valencia-Barrera, R.M., Vega-Maray, A.M., Molnár, T., Fraile, R., 2021. Towards a model of wet deposition of bioaerosols: the raindrop size role. Sci. Total Environ. 767, 145426 https://doi.org/10.1016/j.scitotenv.2021.145426.
Blanco-Alegre, C., Calvo, A.I., Alonso-Blanco, E., Castro, A., Oduber, F., Fraile, R., 2022. Evolution of size-segregated aerosol concentration in NW Spain: a two-step classification to identify new particle formation events. J. Environ. Manage. 114232 https://doi.org/10.1016/j.jenvman.2021.114232.
Bousquet, P.J., Chinn, S., Janson, C., Kogevinas, M., Burney, P., Jarvis, D., 2007a. Geographical variation in the prevalence of positive skin tests to environmental aeroallergens in the European Community Respiratory Health Survey I. Allergy 62 (3), 301–309. https://doi.org/10.1111/j.1398-9995.2006.01293.x.
Bousquet, P.J., Hooper, R., Kogevinas, M., Jarvis, D., Burney, P., Chinn, S., Luezynska, C., Vermeire, P., Kesteloot, H., Bousquet, J., Nowak, D., Prichard, J., de Marco, R., Rijcken, B., Anto, J.M., Alves, J., Boman, G., Abramson, M., Kutin, J., van Bastelaer, F., Neukirch, F., Liard, R., Pin, I., Pison, C., Taytard, A., Magnussen, H., Erich Wichmann, H., Heinrich, J., Gislason, T., Gislason, H.D., Prichard, J., Phil, D., Allwright, S., MacLeod, D., Bugiani, M., Bucca, C., Romano, C., de Marco, R., Cascio, V. Lo, Campello, C., Marinoni, A., Cerveri, I., Casali, L., Kremer, A.M., Crane, J., Lewis, S., Gulsvik, A., Omenaas, E., Sunyer, J., Burgos, F., Tech, R., Castellsague, J., Roca, J., Soriano, J., Tobias, A., Muniozguren, N., Ramos-Gonzalez, J., Capelastegui, A., Castillo, J., Rodriguez-Portal, J., Martinez-Moratalla, J., Almar-Marqnes, E., Maldonado-Pérez, J., Pereira, A., Sfinchez-Ramos, J., Quiros, R., Huerta, I., Payo-Losa, F., Boman, G., Janson, C., Björnsson, E., Rosenhall, L., Norrman, E., Lundbgck, B., Lindholm, N., Plaschke, P., Ackermann-Liebrich, U., Künzli, N., Perruchoud, A., Burr, M., Layzell, J., Russell Hall, B., Harrison, B., Stark, J., Sonia Buist, A., Vollmer, W.M., Osborne, M.L., 2007b. Number of allergens to be tested to assess allergenic sensitization in epidemiologic studies: results of the European Community Respiratory Health Survey I. Clin. Exp. Allergy 37 (5), 780–787. https://doi.org/10.1111/j.1365-2222.2007.02714.x.
Burbach, G.J., Heinzerling, L.M., Edenharter, G., Bachert, C., Bindslev-Jensen, C., Bonini, S., Bousquet, J., Bousquet-Rouanet, L., Bousquet, P.J., Bresciani, M., Bruno, A., Canonica, G.W., Darsow, U., Demoly, P., Durham, S., Fokkens, W.J., Giavi, S., Gjomarkaj, M., Gramiccioni, C., Haahtela, T., Kowalski, M.L., Magyar, P., Muraközi, G., Orosz, M., Papadopoulos, N.G., Röhnelt, C., Stingl, G., Todo-Bom, A., Von Mutius, E., Wiesner, A., Wöhrl, S., Zuberbier, T., 2009. GA2LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe. Allergy 64 (10), 1507–1515. https://doi.org/10.1111/j.1398-9995.2009.02089.x.
Buters, J.T.M., Weichenmeier, I., Ochs, S., Pusch, G., Kreyling, W., Boere, A.J.F., Schober, W., Behrendt, H., 2010. The allergen Bet v 1 in fractions of ambient air deviates from birch pollen counts. Allergy 65 (7), 850–858. https://doi.org/10.1111/j.1398-9995.2009.02286.x.
Buters, J., Prank, M., Sofiev, M., Pusch, G., Albertini, R., Annesi-Maesano, I., Antunes, C., Behrendt, H., Berger, U., Brandao, R., Celenk, S., Galan, C., Grewling, Ł., Jackowiak, B., Kennedy, R., Rantio-Lehtimäki, A., Reese, G., Sauliene, I., Smith, M., Thibaudon, M., Weber, B., Cecchi, L., 2015. Variation of the group 5 grass pollen allergen content of airborne pollen in relation to geographic location and time in season the HIALINE working group. J. Allergy Clin. Immunol. 136 (1), 87–95.e6. https://doi.org/10.1016/j.jaci.2015.01.049.
Caronni, S., Gentili, R., Montagnani, C., Citterio, S., 2021. Subpollen particle release from different species of the invasive allergenic genus *Ambrosia*: the effect of rainwater composition and wind speed. Aerobiologia 37 (4), 785–795. https://doi.org/10.1007/s10453-021-09722-7.
Carslaw, D.C., Ropkins, K., 2012. Openair - an r package for air quality data analysis. Environ. Model. Software 27–28, 52–61. https://doi.org/10.1016/j.envsoft.2011.09.008.
CEN, 2020. Ambient Air - Sampling and Analysis of Airbone Pollen Grains and Fungal Spores for Networks Related to Allergy - Volumetric Hirst Method. CEN.
da Silva, A.R., 2021. Biotools: tools for biometry and apllied statistic in agricultural science. R Package. Versión 4, 2.
Damialis, A., Mohammad, A.B., Halley, J.M., Gange, A.C., 2015. Fungi in a changing world: growth rates will be elevated, but spore production may decrease in future climates. Int. J. Biometeorol. 59 (9), 1157–1167. https://doi.org/10.1007/s00484-014-0927-0.
De Linares, C., Belmonte, J., Canela, M., de la Guardia, C.D., Alba-Sánchez, F., Sabariego, S., Alonso-Pérez, S., 2010. Dispersal patterns of *Alternaria* conidia in Spain. Agric. For. Meteorol. 150 (12), 1491–1500. https://doi.org/10.1016/j.agrformet.2010.07.004.
De Linares, C., Navarro, D., Puigdemunt, R., Belmonte, J., 2022. Airborne Alt a 1. Dynamic and its relationship with the airborne dynamics of *Alternaria* conidia and Pleosporales spores. J. Fungi. 8 (2), 1–12. https://doi.org/10.3390/jof8020125.
Després, V.R., Alex Huffman, J., Burrows, S.M., Hoose, C., Safatov, A.S., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, M.O., Pöschl, U., Jaenicke, R., 2012. Primary biological aerosol particles in the atmosphere: a review. Tellus B Chem. Phys. Meteorol. 64 (1) https://doi.org/10.3402/tellusb.v64i0.15598.
Emberlin, J., 1995. Analysis of allergens on airborne particles: progress and problems. In: Europäisches Pollenflug-Symposium. Duserdorf, Vorträge und Berichte, pp. 48–62.
Emmerson, K.M., Silver, J.D., Thatcher, M., Wain, A., Jones, P.J., Dowdy, A., Newbigin, E.J., Picking, B.W., Choi, J., Ebert, E., Bannister, T., 2021. Atmospheric modelling of grass pollen rupturing mechanisms for thunderstorm asthma prediction. PloS One 16 (4), 1–21. https://doi.org/10.1371/journal.pone.0249488.
Feo Brito, F., Alonso, A.M., Carnés, J., Martín-Martín, R., Fernández-Caldas, E., Galindo, P.A., Alfaya, T., Amo-Salas, M., 2012. Correlation between Alt a 1 levels and clinical symptoms in *Alternaria alternata*- monosensitized patients. J. Investig. Allergol. Clin. Immunol. 22 (3), 154–159.
Fernández, D., Valencia, R.M., Molnár, T., Vega, A., Sagüés, E., 1998. Daily and seasonal variations of *Alternaria* and *Cladosporium* airborne spores in León (North-West, Spain). Aerobiologia 14 (2–3), 215–220. https://doi.org/10.1007/BF02694209.
Fernández-González, D., González-Parrado, Z., Vega-Maray, A.M., Valencia-Barrera, R. M., Camazón-Izquierdo, B., De Nuntiis, P., Mandrioli, P., 2010. *Platanus* pollen allergen, Pla a 1: quantification in the atmosphere and influence on a sensitizing population. Clin. Exp. Allergy 40 (11), 1701–1708. https://doi.org/10.1111/j.1365-2222.2010.03595.x.
Fernández-González, D., Rajo, F.J.R., Parrado, Z.G., Barrera, R.M.V., Jato, V., Grau, S.M., 2011. Differences in atmospheric emissions of Poaceae pollen and Lol p 1 allergen. Aerobiologia 27 (4), 301–309. https://doi.org/10.1007/s10453-011-9199-x.
Fernández-González, M., Guedes, A., Abreu, I., Rodríguez-Rajo, F.J., 2013. Pla a_1 aeroallergen immunodetection related to the airborne *Platanus* pollen content. Sci. Total Environ. 463–464, 855–860. https://doi.org/10.1016/j.scitotenv.2013.06.062.
Fernández-González, D., Vega Maray, A.M., González Parrado, Z., Valencia Barrera, R. M., Gutiérrez, P., De Nuntiis, P., Mandrioli, P., 2019. Are the profilins an important component in the atmosphere? Ole e 2-like panallergen. Aerobiologia 35 (1), 165–175. https://doi.org/10.1007/s10453-018-9548-0.
Fernández-Raga, M., Castro, A., Marcos, E., Fraile, R., 2017. Weather types and rainfall microstructure in Leon. Spain. Int. J. Climatol. 37, 1834–1842. https://doi.org/10.1002/joc.4816.
Forkel, S., Beutner, C., Schröder, S.S., Bader, O., Gupta, S., Fuchs, T., Schön, M.P., Geier, J., Buhl, T., 2021. Sensitization against fungi in patients with airway allergies over 20 years in Germany. Int. Arch. Allergy Immunol. 182 (6), 515–523. https://doi.org/10.1159/000512230.
Gabriel, M.F., Postigo, I., Tomaz, C.T., Martínez, J., 2016. Alternaria alternata allergens: markers of exposure, phylogeny and risk of fungi-induced respiratory allergy. Environ. Int. 89–90, 71–80. https://doi.org/10.1016/j.envint.2016.01.003.
Galán, C., Ariatti, A., Bonini, M., Clot, B., Crouzy, B., Dahl, A., Fernandez-González, D., Frenguelli, G., Gehrig, R., Isard, S., Levetin, E., Li, D.W., Mandrioli, P., Rogers, C.A., Thibaudon, M., Sauliene, I., Skjoth, C., Smith, M., Sofiev, M., 2017. Recommended terminology for aerobiological studies. Aerobiologia 33 (3), 293–295. https://doi.org/10.1007/s10453-017-9496-0.
Galán, C., Smith, M., Damialis, A., Frenguelli, G., Gehrig, R., Grinn-Gofroń, A., Kasprzyk, I., Magyar, D., Oteros, J., Šaulienė, I., Thibaudon, M., Sikoparija, B., 2021. Airborne fungal spore monitoring: between analyst proficiency testing. Aerobiologia 37 (2), 351–361. https://doi.org/10.1007/s10453-021-09698-4.
Garrido-Arandia, M., Silva-Navas, J., Ramírez-Castillejo, C., Cubells-Baeza, N., Gómez-Casado, C., Barber, D., Pozo, J.C., Melendi, P.G., Pacios, L.F., Diáz-Perales, A., 2016. Characterisation of a flavonoid ligand of the fungal protein Alt a 1, scientific reports. Nature 6, 1–9. https://doi.org/10.1038/srep33468.
Gómez-Casado, C., Murua-García, A., Garrido-Arandia, M., González-Melendi, P., Sánchez-Monge, R., Barber, D., Pacios, L.F., Díaz-Perales, A., 2014. Alt a 1 from *Alternaria* interacts with PR5 thaumatin-like proteins. FEBS Lett. 588 (9), 1501–1508. https://doi.org/10.1016/j.febslet.2014.02.044.
Graversen, S., 1979. Fungi as a cause of allergic disease. Allergy 34, 135–154. https://doi.org/10.1001/jama.1995.03520250039026.
Grewling, Ł., Bogawski, P., Kryza, M., Magyar, D., Šikoparija, B., Skjøth, C.A., Udvardy, O., Werner, M., Smith, M., 2019. Concomitant occurrence of anthropogenic air pollutants, mineral dust and fungal spores during long-distance transport of ragweed pollen. Environ. Pollut. 254 https://doi.org/10.1016/j.envpol.2019.07.116.
Grewling, Ł., Bogawski, P., Szymańska, A., Nowak, M., Kostecki, Ł., Smith, M., 2020. Particle size distribution of the major *Alternaria* alternata allergen, Alt a 1, derived from airborne spores and subspore fragments. Fungal Biol. 124 (3–4), 219–227. https://doi.org/10.1016/j.funbio.2020.02.005.
Grewling, Ł., Magyar, D., Chłopek, K., Grinn-Gofroń, A., Gwiazdowska, J., Siddiquee, A., Ianovici, N., Kasprzyk, I., Wójcik, M., Laffersová, J., Majkowska-Wojciechowska, B., Myszkowska, D., Rodinkova, V., Bortnyk, M., Malkiewicz, M., Piotrowska-Weryszko, K., Sulborska-Różycka, A., Rybniček, O., Ščevková, J., Šikoparija, B., Skjøth, C.A., Smith, M., Bogawski, P., 2022. Bioaerosols on the atmospheric super highway: An example of long distance transport of *Alternaria* spores from the Pannonian Plain to Poland. Sci. Total Environ. 819 https://doi.org/10.1016/j.scitotenv.2022.153148.
Grinn-Gofroń, A., Bosiacka, B., 2015. Effects of meteorological factors on the composition of selected fungal spores in the air. Aerobiologia 31 (1), 63–72. https://doi.org/10.1007/s10453-014-9347-1.

Grinn-Gofroń, A., Strzelczak, A., 2008. Artificial neural network models of relationships between *Alternaria* spores and meteorological factors in Szczecin (Poland). Int. J. Biometeorol. 52 (8), 859–868. https://doi.org/10.1007/s00484-008-0182-3.

Grinn-Gofroń, A., Nowosad, J., Bosiacka, B., Camacho, I., Pashley, C., Belmonte, J., De Linares, C., Ianovici, N., Manzano, J.M.M., Sadyś, M., Skjøth, C., Rodinkova, V., Tormo-Molina, R., Vokou, D., Fernández-Rodríguez, S., Damialis, A., 2019. Airborne *Alternaria* and *Cladosporium* fungal spores in Europe: forecasting possibilities and relationships with meteorological parameters. Sci. Total Environ. 653 (October 2018), 938–946. https://doi.org/10.1016/j.scitotenv.2018.10.419.

Gutiérrez-Rodríguez, A., Postigo, I., Guisantes, J.A., Suñén, E., Martínez, J., 2011. Identification of allergens homologous to Alt a 1 from *Stemphylium botryosum* and *Ulocladium botrytis*. Medical Mycol. 49 (8), 892–896. https://doi.org/10.3109/13693786.2011.576350.

Hassett, M.O., Fischer, M.W.F., Money, N.P., 2015. Mushrooms as rainmakers: how spores act as nuclei for raindrops. PloS One 10 (10), 1–10. https://doi.org/10.1371/journal.pone.0140407.

Hatzipapas, P., Kalosaka, K., Dara, A., Christias, C., 2002. Spore germination and appressorium formation in the entomopathogenic *Alternaria alternata*. Mycol. Res. 106 (11), 1349–1359. https://doi.org/10.1017/S0953756202006792.

Hirst, J.M., 1952. An automatic volumetric spore trap. Ann. Appl. Biol. 39 (2), 257–265.

Hyvönen, S., Junninen, H., Laakso, L., Dal Maso, M., Grönholm, T., Bonn, B., Keronen, P., Aalto, P., Hiltunen, V., Pohja, T., Launiainen, S., Hari, P., Mannila, H., Kulmala, M., 2005. A look at aerosol formation using data mining techniques. Atmospheric Chem. Phys. 5 (12), 3345–3356. https://doi.org/10.5194/acp-5-3345-2005.

Ibarrola, I., Suárez-Cervera, M., Arilla, M.C., Martínez, A., Monteseirín, J., Asturias, J.A., 2004. Production profile of the major allergen Alt a 1 in *Alternaria alternata* cultures. Ann. Allergy Asthma Immunol. 93 (6), 589–593. https://doi.org/10.1016/S1081-1206(10)61268-9.

Idrose, N.S., Dharmage, S.C., Lowe, A.J., Lambert, K.A., Lodge, C.J., Abramson, M.J., Douglass, J.A., Newbigin, E.J., Erbas, B., 2020. A systematic review of the role of grass pollen and fungi in thunderstorm asthma. Environ. Res. 181 (November 2019), 108911 https://doi.org/10.1016/j.envres.2019.108911.

ITACYL, AEMET, 2013. Átlas Agroclimático de Castilla y León. 1o. Catálogo de Publicaciones de la Administración General del Estado. https://doi.org/10.2307/j.ctv6gqvd9.20.

Kasprzyk, I., Rodinkova, V., Šaulienė, I., Ritenberga, O., Grinn-Gofron, A., Nowak, M., Sulborska, A., Kaczmarek, J., Weryszko-Chmielewska, E., Bilous, E., Jedryczka, M., 2015. Air pollution by allergenic spores of the genus *Alternaria* in the air of central and eastern Europe. Environ. Sci. Pollut. Res. 22 (12), 9260–9274. https://doi.org/10.1007/s11356-014-4070-6.

Kassambara, A., Mundt, F., 2016. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R Package version 1.0.3.

Ketchen, D.J., Shook, C.L., 1996. The application of cluster analysis in strategic management research: an analysis and critique. Strateg. Manag. J. 17 (6), 441–458. http://www.jstor.org/stable/2486927.

Kustrzeba-Wójcicka, I., Siwak, E., Terlecki, G., Wolańczyk-Mędrala, A., Mędrala, W., 2014. Alternaria alternata and its allergens: a comprehensive review. Clin Rev Allergy Immunol 47 (3), 354–365. https://doi.org/10.1007/s12016-014-8447-6.

Lara, B., Rojo, J., Costa, A.R., Burgos-Montero, A.M., Antunes, C.M., Pérez-Badia, R., 2023. Atmospheric pollen allergen load and environmental patterns in central and southwestern Iberian Peninsula. Sci. Total Environ. 858 (September 2022), 159630 https://doi.org/10.1016/j.scitotenv.2022.159630.

Lawrence, D.P., Rotondo, F., Gannibal, P.B., 2016. Biodiversity and taxonomy of the pleomorphic genus *Alternaria*. Mycol. Prog. 15 (3) https://doi.org/10.1007/s11557-015-1144-x.

Logrieco, A., Bottalico, A., Mulé, G., Moretti, A., Perrone, G., 2003. Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. Eur. J. Plant Pathol. 109 (7), 645–667. https://doi.org/10.1023/A:1026033021542.

Mandrioli, P., Comtois, P., Levizzani, V., 1998. In: Mandrioli, P., Comtois, P., Levizzani, V. (Eds.), Methods in Aerobiology. Pitagora Editrice S.r.l, Bologna.

Martínez-Quesada, J., Gutiérrez, A., Postigo, I., Cardona, G., Guisantes, J., 2006. Variability of Alt a 1 expression by different strains of *Alternaria alternata*. J. Investig. Allergol. Clin. Immunol. 16 (5), 279–282.

McCartney, H.A., Schmechel, D., Lacey, M.E., 1993. Aerodinamic diameter of conidia of *Alternaria* species. Plant Pathol. 42, 280–286.

Moreno-Grau, S., Elvira-Rendueles, B., Moreno, J., García-Sánchez, A., Vergara, N., Asturias, J.A., Arilla, M.C., Ibarrola, I., Seoane-Camba, J.A., Suárez-Cervera, M., 2006. Correlation between *Olea europaea* and *Parietaria judaica* pollen counts and quantification of their major allergens Ole e 1 and Par j 1-Par j 2. Ann. Allergy Asthma Immunol. 96 (6), 858–864. https://doi.org/10.1016/S1081-1206(10)61350-6.

Nowakowska, M., Wrzesińska, M., Kamiński, P., Szczechura, W., Lichocka, M., Tartanus, M., Kozik, E.U., Nowicki, M., 2019. *Alternaria brassicicola* – Brassicaceae pathosystem: insights into the infection process and resistance mechanisms under optimized artificial bio-assay. Eur. J. Plant Pathol. 153 (1), 131–151. https://doi.org/10.1007/s10658-018-1548-y.

O'Connor, D.J., Sadyś, M., Skjøth, C.A., Healy, D.A., Kennedy, R., Sodeau, J.R., 2014. Atmospheric concentrations of *Alternaria, Cladosporium, Ganoderma* and *Didymella* spores monitored in Cork (Ireland) and Worcester (England) during the summer of 2010. Aerobiologia 30 (4), 397–411. https://doi.org/10.1007/s10453-014-9337-3.

Oksanen, A.J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., Hara, R.B.O., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., 2020. Vegan: community ecology package. R-Package 2395–2396.

Picornell, A., Rojo, J., Trigo, M.M., Ruiz-Mata, R., Lara, B., Romero-Morte, J., Serrano-García, A., Pérez-Badia, R., Gutiérrez-Bustillo, M., Cervigón-Morales, P., Ferencova, Z., Morales-González, J., Sánchez-Reyes, E., Fuentes-Antón, S., Sánchez-Sánchez, J., Dávila, I., Oteros, J., Martínez-Bracero, M., Galán, C., García-Mozo, H., Alcázar, P., Fernández, S., González-Alonso, M., Robles, E., de Zabalza, A.P., Ariño, A.H., Recio, M., 2022. Environmental drivers of the seasonal exposure to airborne *Alternaria* spores in Spain. Sci. Total Environ. 823 https://doi.org/10.1016/j.scitotenv.2022.153596.

Plaza, M.P., Alcázar, P., Hernández-Ceballos, M.A., Galán, C., 2016. Mismatch in aeroallergens and airborne grass pollen concentrations. Atmospheric Environ. 144, 361–369. https://doi.org/10.1016/j.atmosenv.2016.09.008.

Plaza, M.P., Alcázar, P., Velasco-Jiménez, M.J., Galán, C., 2017. Aeroallergens: a comparative study of two monitoring methods. Aerobiologia 33 (3), 363–373. https://doi.org/10.1007/s10453-017-9475-5.

Postigo, I., Gutiérrez-Rodríguez, A., Fernández, J., Guisantes, J.A., Suñŕn, E., Martínez, J., 2011. Diagnostic value of Alt a 1, fungal enolase and manganese-dependent superoxide dismutase in the component-resolved diagnosis of allergy to Pleosporaceae. Clin. Exp. Allergy 41 (3), 443–451. https://doi.org/10.1111/j.1365-2222.2010.03671.x.

Pulimood, T.B., Corden, J.M., Bryden, C., Sharples, L., Nasser, S.M., 2007. Epidemic asthma and the role of the fungal mold *Alternaria alternata*. J. Allergy Clin. Immunol. 120 (3), 610–617. https://doi.org/10.1016/j.jaci.2007.04.045.

Rapiejko, P., Lipiec, A., Wodjas, A., Jurkiewicz, D., 2004. Threshold pollen concentration necessary to evoke allergic symptoms. Int. Rev. Allergol. 10 (3), 91–94.

Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, A., Firth, D., 2023. Support Functions and Datasets for Venables and Ripley's MASS.

Rodriguez, M.Z., Comin, C., Casanova, D., Bruno, M., Amancio, D., Costa, L., Rodrigues, F., 2019. Clustering algorithms: a comparative approach. PloS One 14 (1), e0210236. https://doi.org/10.1371/journal.pone.0210236.

Rodríguez, D., Tabar, A.I., Castillo, M., Martínez-Gomariz, M., Dobski, I.C., Palacios, R., 2021. Changes in the sensitization pattern to *Alternaria alternata* allergens in patients treated with alt a 1 immunotherapy. J. Fungi. 7 (11) https://doi.org/10.3390/jof7110974.

Rodríguez-Fernández, A., Oteros, J., Vega-Maray, A.M., Valencia-Barrera, R.M., Galán, C., Fernández-González, D., 2022. How to select the optimal monitoring locations for an aerobiological network: a case of study in central northwest of Spain. Sci. Total Environ. 827, 154370 https://doi.org/10.1016/j.scitotenv.2022.154370.

Rodríguez-Fernández, A., Blanco-Alegre, C., Vega-Maray, A.M., Valencia-Barrera, R.M., Molnár, T., Fernández-González, D., 2023. Effect of prevailing winds and land use on *Alternaria* airborne spore load. J. Environ. Manage. 332, 117414 https://doi.org/10.1016/j.jenvman.2023.117414.

Rodríguez-Rajo, F.J., Iglesias, I., Jato, V., 2005. Variation assessment of airborne *Alternaria* and *Cladosporium* spores at different bioclimatical conditions. Mycol. Res. 109 (4), 497–507. https://doi.org/10.1017/S0953756204001777.

Rodríguez-Rajo, F.J., Jato, V., González-Parrado, Z., Elvira-Rendueles, B., Moreno-Grau, S., Vega-Maray, A., Fernández-González, D., Asturias, J.A., Suárez-Cervera, M., 2011. The combination of airborne pollen and allergen quantification to reliably assess the real pollinosis risk in different bioclimatic areas. Aerobiologia 27 (1), 1–12. https://doi.org/10.1007/s10453-010-9170-2.

Roever, C., Raabe, N., Luebke, K., Ligges, U., Szepannek, G., Zent-graf, M., Meyer, D., 2023. KlarR: classification and visualization. R package. Version 1.7-2. Available online: https://cran.r-project.org/web/packages/klaR/index.html. (Accessed 1 May 2023).

Suanno, C., Sandrini, S., Aloisi, I., De Nuntiis, P., Facchini, M.C., Del Duca, S., Fernández-González, D., 2022. Airborne pollen, allergens, and proteins: a comparative study of three sampling sethods. Sustainability (Switzerland). 14 (19) https://doi.org/10.3390/su141911825.

Takahashi, Y., Ohashi, T., Nagoya, T., Sakaguchi, M., Yasueda, H., Nitta, H., 2001. Possibility of real-time measurement of an airborne *Cryptomeria japonica* pollen allergen based on the principle of surface plasmon resonance. Aerobiologia 17 (4), 313–318. https://doi.org/10.1023/A:1013002001583.

Teifoori, F., Shams-Ghahfarokhi, M., Razzaghi-Abyaneh, M., Martinez, J., 2019. Gene profiling and expression of major allergen Alt a 1 in *Alternaria alternata* and related members of the Pleosporaceae family. Rev. Iberoam. Micol. 36 (2), 66–71. https://doi.org/10.1016/j.riam.2018.01.006.

Thomma, B.P.H.J., 2003. *Alternaria* spp.: from general saprophyte to specific parasite. Mol. Plant Pathol. 4 (4), 225–236. https://doi.org/10.1046/j.1364-3703.2003.00173.x.

Twaroch, T.E., Arcalís, E., Sterflinger, K., Stöger, E., Swoboda, I., Valenta, R., 2012. Predominant localization of the major *Alternaria* allergen Alt a 1 in the cell wall of airborne spores. J. Allergy Clin. Immunol. 129 (4), 1148–1149. https://doi.org/10.1016/j.jaci.2011.10.008.

Uria-Tellaetxe, I., Carslaw, D.C., 2014. Conditional bivariate probability function for source identification. Environ. Model. Software 59, 1–9. https://doi.org/10.1016/j.envsoft.2014.05.002.

Vara, A., Fernández-González, M., Aira, M.J., Rodríguez-Rajo, F.J., 2016. Oleaceae cross-reactions as potential pollinosis cause in urban areas. Sci. Total Environ. 542, 435–440. https://doi.org/10.1016/j.scitotenv.2015.10.107.

Wang, Q., Nakamura, S., Lu, S., Xiu, G., Nakajima, D., Suzuki, M., Sakamoto, K., Miwa, M., 2012. Release behavior of small sized daughter allergens from *Cryptomeria japonica* pollen grains during urban rainfall event. Aerobiologia 28 (1), 71–81. https://doi.org/10.1007/s10453-011-9212-4.

Wang, X., Li, X., Ma, R., Li, Y., Wang, W., Huang, H., Xu, C., An, Y., 2018. Quadratic discriminant analysis model for assessing the risk of cadmium pollution for paddy fields in a county in China. Environ. Pollut. 236, 366–372. https://doi.org/10.1016/j.envpol.2018.01.088.

Wei, T., Simko, V., 2017. Corrplot: visualization of a correlation matrix. R package. Version 0, 84.

Woudenberg, J.H.C., Groenewald, J.Z., Binder, M., Crous, P.W., 2013. *Alternaria* redefined. Stud. Mycol. 75, 171–212. https://doi.org/10.3114/sim0015.

Woudenberg, J.H.C., Seidl, M.F., Groenewald, J.Z., de Vries, M., Stielow, J.B., Thomma, B.P.H.J., Crous, P.W., 2015. *Alternaria* section *Alternaria*: species, formae speciales or pathotypes? Stud. Mycol. 82, 1–21. https://doi.org/10.1016/j.simyco.2015.07.001.

Yamamoto, N., Bibby, K., Qian, J., Hospodsky, D., Rismani-Yazdi, H., Nazaroff, W.W., Peccia, J., 2012. Particle-size distributions and seasonal diversity of allergenic and pathogenic fungi in outdoor air. ISME J. 6 (10), 1801–1811. https://doi.org/10.1038/ismej.2012.30.

Zureik, M., Neukirch, C., Leynaert, B., Liard, R., Bousquet, J., Neukirch, F., 2002. Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. Brit. Med. J. 325 (7361), 411–414. https://doi.org/10.1542/peds.112.s2.480a.