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Research article

Effect of prevailing winds and land use on *Alternaria* airborne spore load

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ABSTRACT

Alternaria spores are a common component of the bioaerosol. Many *Alternaria* species are plant pathogens, and their conidia are catalogued as important aeroallergens. Several aerobiological studies showing a strong relationship between concentrations of airborne spore and meteorological parameters have consequently been developed. However, the *Alternaria* airborne load variation has not been thoroughly investigated because it is difficult to assess their sources, as they are a very common and widely established phytopathogen. The objective of this study is to estimate the impact of vegetation and land uses as potential sources on airborne spore load and to know their influence, particularly, in cases of long-medium distance transport. The daily airborne spore concentration was studied over a 5-year period in León and Valladolid, two localities of Castilla y León (Spain), with differences in their bioclimatic and land use aspects. Moreover, the land use analysis carried out within a 30 km radius of each monitoring station was combined with air mass data in order to search for potential emission sources. The results showed a great spatial variation between the two areas, which are relatively close to each other. The fact that the spore concentrations recorded in Valladolid were higher than those in León was owing to prevailing winds originating from large areas covered by cereal crops, especially during the harvest period. However, the prevailing winds in León came from areas dominated by forest and shrubland, which explains the low airborne spore load, since the main *Alternaria* sources were the grasslands located next to the trap. Furthermore, the risk days in this location presented an unusual wind direction. This study reveals the importance of land cover and wind speed and direction data for establishing potential airborne routes of spore transport in order to improve the *Alternaria* forecasting models. The importance of conducting *Alternaria* aerobiological studies at a local level is also highlighted.

1. Introduction

Fungal material is one of the most abundant components of primary biologic aerosol particles (PBAP) (Després et al., 2012; Yamamoto et al., 2012). It is composed mainly of spores (Graversen, 1979; Hassett et al., 2015), which are typically grouped into the coarse particulate matter fractions (particles >1 μm) owing to their size range (1–100 μm), having most of them a diameter around 10 μm (Elbert et al., 2007; Mandrioli et al., 1998).

Alternaria is a cosmopolitan genus of more than 300 species (Lawrence et al., 2016; Woudenberg et al., 2013, 2015), signifying that its spores are found in most environments, especially in rural areas (Apangu et al., 2020), and it is one of the most abundant airborne fungal taxa (Banchi et al., 2018; Dietzel et al., 2019; Fröhlich-Nowoisky et al., 2009; Woo et al., 2018). Many species are phytopathogens that produce important yield losses in the agricultural sector, affecting a large variety of crops and post-harvest fruits (Abuley and Nielsen, 2017; Logrieco et al., 2003; Meena and Samal, 2019; Nowakowska et al., 2019; Thomma, 2003). Furthermore, *Alternaria* is clinically significant since it is listed as an important airborne allergen source and is linked primarily to respiratory disorders, particularly in children (Gabriel et al., 2016; Hernandez-Ramírez et al., 2021; Kustrzeba-Wójcicka et al., 2014; Pulimood et al., 2007). It is estimated that the average percentage of sensitization to *Alternaria* in the world is 4.4%, although there is a great variation among countries. In Europe, the prevalence of sensitization to *Alternaria* is 6.1%, although in Spain these percentages range from 0.2%

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to 1.9%, depending on the city (Bousquet et al., 2007a, 2007b; Forkel et al., 2021).

Many studies concerning *Alternaria* airborne conidia have consequently been conducted in different areas, and have established that the main parameters that control the presence of airborne conidia, whose dispersion is favored in warm dry periods, are temperature, precipitation and relative humidity (Forkel et al., 2021; Grinn-Gofroń and Bosiacka, 2015; O'Connor et al., 2014; Recio et al., 2011; Rodríguez-Rajo et al., 2005). The main spore season (MSS) generally has two main types of behaviour, with an alternation between a high concentration period and either a lower or an absent concentration period (Grinn-Gofroń et al., 2019). Northern locations in Europe similarly have a well-defined MSS during summer months, with peak days usually occurring between the end of July and August (Apangu et al., 2020; Corden et al., 2003; Olsen et al., 2020, 2020evková et al., 2016; Skjøth et al., 2012; Tomassetti et al., 2012). However, airborne conidia are present during longer periods in Mediterranean areas (Skjøth et al., 2016), and the MSS is usually split into two seasons: spring and autumn (Damialis and Gioulekas, 2006; Marchesi, 2020; Picornell et al., 2022). The Iberian Peninsula has a great variety of both MSS patterns and concentrations: the south-western territories have a higher concentration and two peaks, with a drop in concentration in August, while northern locations have only one peak in summer and lower concentrations (Aira et al., 2013; De Linares et al., 2010; Fernández et al., 1998; Picornell et al., 2022).

Nevertheless, the behaviour of airborne spore is a complex phenomenon and cannot be explained solely by meteorological parameters (Grinn-Gofroń and Bosiacka, 2015; Sadyś et al., 2014). *Alternaria* dispersion has been reported to be mainly local, and the greatest amount of conidia are correlated to the harvest period (Skjøth et al., 2012). It is for this reason that the vegetation around the city plays an important role in *Alternaria* presence that can explain the differences in concentrations among places in close proximity to each other. Despite this, long distance transport episodes have been associated with sudden days of high concentrations, owing to the arrival of air masses coming from potential source areas (Apangu et al., 2020; Fernández-Rodríguez et al., 2015; Grewling et al., 2022; Grinn-Gofroń et al., 2020; Olsen et al., 2020; Sadyś et al., 2015; Skjøth et al., 2012). Furthermore, the effect of wind speed on *Alternaria* concentrations is still not clear, since their correlation is usually low, especially in inland territories (Fernández et al., 1998; Filali Ben Sidel et al., 2015; Grinn-Gofroń and Bosiacka, 2015; Recio et al., 2011; Sabariego et al., 2012; Sánchez-Reyes et al., 2016). These results may be due to the treatment of wind speed data without considering wind direction, persistence and emission sources together.

For all of the aforementioned reasons, the objective of this study is to determine the impact of land use on the presence of *Alternaria* airborne spores, in addition to discovering which types of vegetation are the most important emission sources. Two relatively close cities with a similar climate but with different land uses have, therefore, been chosen for this purpose. The study also seeks to clarify the impact of air mass on the *Alternaria* airborne spore load and to establish the potential airborne spore transportation pathways that increase concentrations of *Alternaria* in the atmosphere, thus resulting in risk days for the allergic population.

2. Materials and method

2.1. Study area

The study was conducted in León and Valladolid (Fig. 1), which are located 140 km from each other. Both cities are in the region of Castilla y León, which is located in the central northwest area of Spain, and is one of the most important agricultural areas in the country. Although both cities are characterised by having a Continental Mediterranean climate, they present bioclimatic differences, along with different vegetation and orography, which divide the territory into two aerobiological areas according to airborne pollen behaviour. Leon is a small city located in the north of the region (42° 36′ N, 05° 35′ W and 819 m a.s.l) with a mean temperature of around 11.4 °C and a mean annual precipitation of 544 mm. The winters are long and cold, with the mean temperature of the coldest quarter being 4.1 °C. The average temperature in the warmest quarter is, however, 18.9 °C, with a mean precipitation of 96 mm. Valladolid (41° 64′ N, 04° 73′ W and 713 m a.s.l) is, in contrast, the largest city in the region. It is located on the inner plateau where the average temperature is above 12.4 °C and the mean annual precipitation is 420 mm. The winters are cold, with a mean temperature of over 4.7 °C, and the summers are hot and dry, characterised by average temperatures of over 20.4 °C in the warmest quarter and low precipitations (65 mm) (Rodríguez-Fernández et al., 2022).

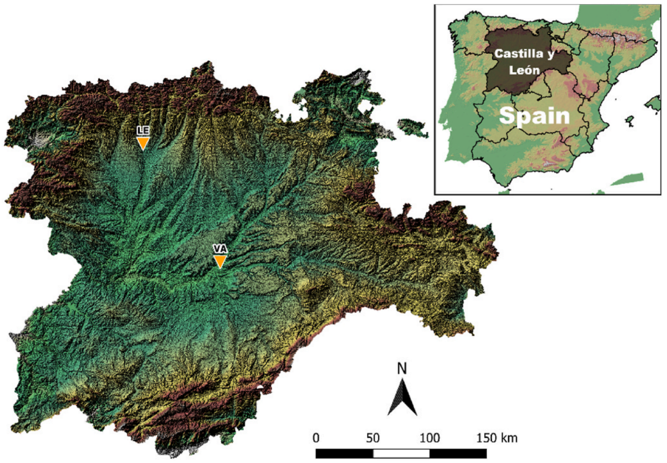


Fig. 1. Locations of aerobiological stations in León (LE) and Valladolid (VA) in Castilla y León.

2.2. Spore sampling

The *Alternaria* spore concentrations were monitored in León and Valladolid from 2016 to 2020 using Hirst type monitoring station (Hirst, 1952) 7-day recording volumetric traps (Logrieco et al., 2003). The trap in León is located on the terrace of the Faculty of Veterinary Science (University of León), while the monitoring station in Valladolid is located on the terrace of the Health Department building. Both traps are installed over 15 m above ground level in a place that is free from any obstacles that might hinder the arrivals of air masses and bioaerosol emission sources. The flow was adjusted to 10 L min^{-1} using a handheld Lanzoni flow-meter with air flow resistance. These traps are part of the Castilla y León Aerobiological network (RACyL), and now follow the methodology proposed by CEN legislation EN 16868:2019 (CEN, 2019), although they previously followed the methodologies proposed by the Spanish Aerobiology Network (REA) (Galán et al., 2007) and the minimum recommendations of the European Aerobiology Society (EAS) (Galán et al., 2014). In the period selected, the monitoring stations were working continuously, and the database was complete at 96%, signifying that imputation data procedures were not necessary.

The samples were analysed under a light microscope at 400 magnifications using two longitudinal transects in the effective collecting area. The area analysed equates to 8% of the slide examined, which is more than the 5% recommended (Galán et al., 2021). All the samples were analysed by the same technician in order to reduce methodological bias owing to spore identification. The spore concentration and Annual Spore Integral (ASIn) were expressed as spores/m^3 and spore/day × m^3, respectively, using the terminology recommend for aerobiological studies (Galán et al., 2017). Moreover, the start and end of the Main Spore Season (MSS) were defined as 10 consecutive days with at least 5 spores/m^3 (Galán et al., 2001). As suggested by Graversen (1979), days with a concentration of ≥100 spores/m^3 were catalogued as risk days, since this is the threshold for evoking respiratory symptomatology in *Alternaria* sensitised individuals. The Wicolxon test was applied in order to ensure that the two locations had significant differences with respect to the spore concentration and the length of the MSS. All aerobiological calculations were made using the "Aerobiology" R-package (Rojo et al., 2019) in R statistical software (http://www.r-project.org/).

2.3. *Land cover data*

A 30 km radius of land use surrounding the monitoring stations was analysed. The choice of this distance was based on previous research on the representativeness of pollen and spore emission sources using volumetric samplers (Apangu et al., 2020; Avolio et al., 2008; O'Connor et al., 2014; Oteros et al., 2017; Rojo et al., 2016; Skjøth et al., 2010). The land use data were obtained from Castilla y León crops and natural land maps (MCSNCyL). This land cover layer system uses satellite imagery in the Copernicus programme and has a GSD (ground sampling distance) spatial resolution of 10 m, thus making it ideal for land use monitoring in this region, in which the average plot is 2.4 ha (Paredes-Gómez et al., 2020). The land use data are available at: http://mcsncyl.itacyl.es/en/inicio. The cover classes were grouped into 19 main groups for a better visualization of the map, taking into account crop features in the region.

2.4. *Analysis of air masses*

Meteorological parameters of wind direction and wind speed during the study period (2016–2020) were provided by the Spanish Meteorological Agency (AEMET). The meteorological stations in León and Valladolid are located 6 km and 3 km from the spore traps, respectively. All calculations related to wind parameters were made using Open air R-package (Carslaw, 2019; Carslaw and Ropkins, 2012). The analysis of the prevailing winds (wind roses) for both localities was carried out using data from the entire study period. Furthermore, in order to discover the origin of potential *Alternaria* emission sources: I) Bivariate Polar Plots (Polar plots) and Conditional Probability function plots (CPF) were applied by considering the MSS periods. The 90th interval concentration percentile for the CPF was chosen because the spore concentration is usually low (Uria-Tellaetxe and Carslaw, 2014). If these methods did not clearly show the direction of potential emission sources, then: II) the origin and sources of air masses were obtained by applying the HYSPLIT4 model (Hybrid Single Particle Lagrangian Integrated Trajectory) (Draxler and Rolph, 2012). This model was used to compute 48 h back trajectories on every day selected, at an arrival altitude of 200 m a.g.l. Over the sampling point (Damialis et al., 2017).

The model was run with meteorological data: I) from the Global Data Assimilation System (GDAS) archives (spatial resolution of 0.5°) until 2019, and II) from meteorological data from the Global Forecast System (GFS) (spatial resolution of 0.25°) from 2019 to 2020. Moreover, the number of hours of each air mass within a 60 km radius was considered in order to analyse the possible influence of air mass stability on the *Alternaria* concentration. The origins of the prevailing air masses and transport pathways were subsequently classified in four groups (NW, SW, SE and NE). The criterion employed to assign these aspects to a particular group was the sector in which the air mass spent most of its time (Blanco- Alegre et al., 2019) within a 60 km radius.

3. Results

Regarding the behaviour of *Alternaria* airborne spores, the two locations under examination differed greatly (Fig. 2). There was no pronounced increase in the spore concentration in León, and its average concentration had many small peaks and a slight drop in August.

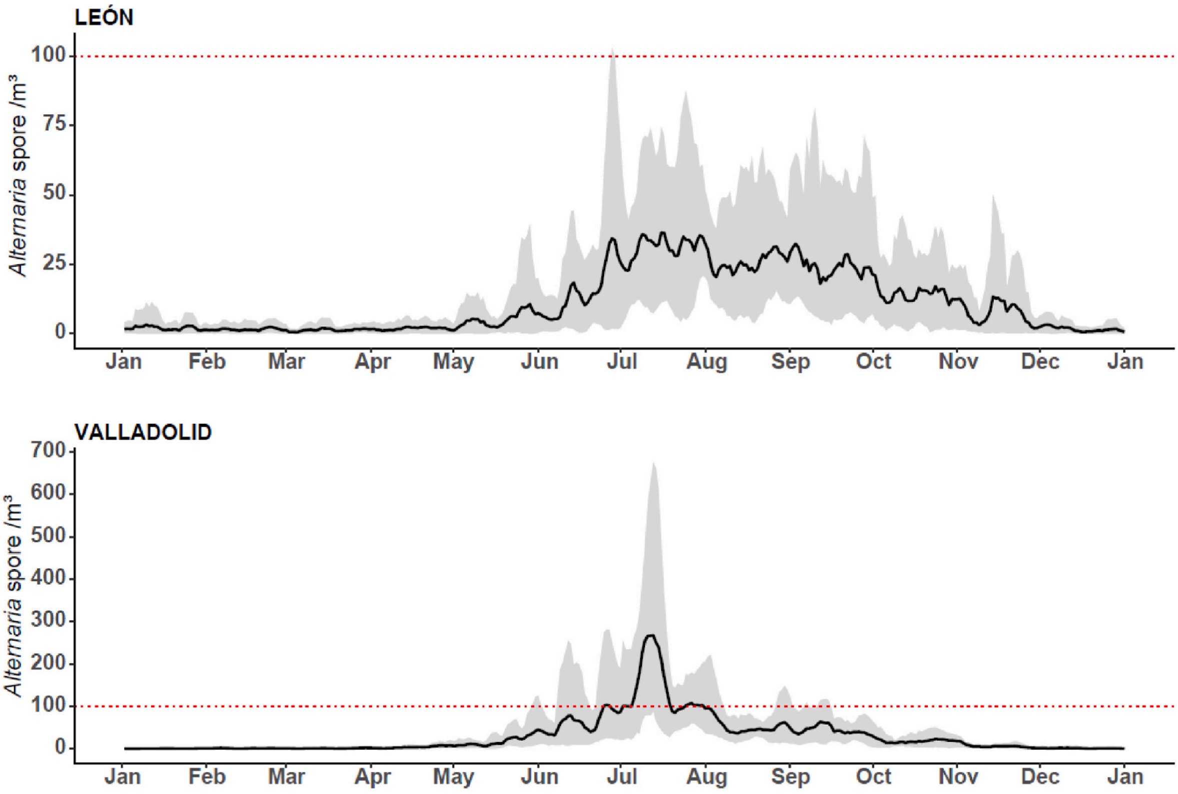


Fig. 2. Maximum values (grey area), minimum values (white area below grey) and daily average concentration (black line) of *Alternaria* spore as a result of 5-day moving average in the sampling period (2016–2020). Red dashed line highlights the *Alternaria* spore concentration (100 spores/m^3) because the Y-axis represents different values in each plot.

Moreover, León had low maximum values, and spore concentrations of over 100 spores/m^3 were rare (values resulting from the 5-day moving average). This location also had low minimum values during the period studied, and these were greater than 25 spores/m^3. However, Valladolid had a clear pattern of spore concentration, with high values in mid-July and a drastic fall in August. The maximum values resulting from the 5-day moving average in Valladolid reached 700 spores/m^{3,} and the minimum concentrations had values close to 100 spores/m^3.

The ASIn was much higher in Valladolid, and was in fact almost double that of León, in all the years analysed (Table 1), and there were significant differences (Wilcoxon test value 0.016). The lowest concentration was recorded in 2016 for both sampling points, whereas the year with the greatest ASIn varied depending on the station, and was 2018 in León (5095 spores) and 2017 in Valladolid (15,149 spores). Valladolid had the greatest number of risk days (38), which matched the years in which the highest values for ASIn were recorded (2017 and 2018); the lowest number of these days was, however in 2016. The greatest number of risk days in León, meanwhile, occurred in 2019, with a total of 5. Moreover, León had two years without risk days (2016 and 2017).

Differences between the cities as regards the MSS length were also observed (Wilcoxon test value 0.036). The MSS was earlier, longer and more homogeneous in Valladolid, always starting in May during the years analysed, whereas the onset of the MSS in León varied depending on the year, occurring at the end of May, or in June or early July (Table 1). The end of the MSS tended to occur between October and November at both stations, although it ended earlier in León in 2020. With regard to the peak days, León did not have a clear pattern, and they could occur at any time during the MSS. In 2020, the peak day occurred outside the MSS in León. However, this was an isolated concentration peak, and the second day with the highest concentration (112 spores/m^3) was on 29th September, which was in the MSS. All the peak dates in Valladolid were, meanwhile, around mid-July, varying by a few days according to the year.

The wind data observed for León and Valladolid in the period studied (2016–2020) is depicted in the wind roses shown in Fig. 3 A. The average wind speed was low on both sites, and was 1.62 m s $^{-1}$ in León and 1.32 m s $^{-1}$ in Valladolid. The percentage of wind calm was similarly null in León and very low (0.3%) in Valladolid. León was dominated by northwest flow winds, although winds from the west and southwest were also common. The highest frequencies of low wind velocities (>0–2 m s $^{-1}$) were observed in the northwest, whereas the highest wind velocities (6–9 m s $^{-1}$) were recorded in the southwest quadrant. However, these speeds were unusual in this area and presented a low frequency. The southeast quadrant obtained the lowest frequency of counts in León. Valladolid showed prevailing winds came from northerly directions, although northeast and northwest flows were also common. These directions had a high frequency of wind speeds of between >0 and 2 m s $^{-1}$. Winds from the southwest were also counted with great frequency. In this case, wind speeds with a range of between 2 and 4 m s $^{-1}$ were usual, together with low wind speeds. The highest wind speed was registered (6–7 m s $^{-1}$) in this quadrant, although this speed was unusual (<1%). Winds from the east, south and southeast were not common in Valladolid during the period studied.

Table 1

Main spore season (MSS) characteristic for *Alternaria* in León (LE) and Valladolid (VA) during the sampling period (2016–2020). Annual Spore Integral (ASIn).

Station	Year	Start-End MSS date	Peak date	Peak value (spores m^{-3})	ASIn (spores/day m^{-3})	Risk days (≥100 spores m^{-3})
LE	2016	02-Jul/08-Oct	20/Sep	70	2210	0
LE	2017	20-May/03-Nov	27/May	74	3115	0
LE	2018	14-Jul/27-Oct	09/Sep	155	5095	3
LE	2019	25-Jun/12-Oct	27/Jun	120	4416	5
LE	2020	19-Jul/18-Sep	15/Nov	118	4751	3
VA	2016	14-May/03-Nov	12/Jul	134	4819	4
VA	2017	13-May/02-Nov	13/Jul	812	15,149	38
VA	2018	26-May/29-Oct	09/Jul	522	13,899	38
VA	2019	05-May/05-Nov	16/Jul	399	8395	27
VA	2020	18-May/03-Oct	10/Jul	239	7497	11

The land cover within a 30 km radius of the monitoring stations is represented in Fig. 4, while the land use percentages are shown in supplementary Table A1. There was a predominance of woodland (15.5%), shrubs (12.7%) and pastures (10.3%) in León, while cereal crops represented only 7.9% of the total area. Quadrant 1 (north-east) was dominated by another woodland class, in which broad leaf and conifer forests are clustered together, representing up to 50.6% of the total of the area. Quadrant 4 (north-west) also had a significant percentage of cover of these vegetation types (35.3%), along with a significant area cover by scrubland (28.7%). The southern areas (quadrants 2 and 3) were mostly dominated by agricultural land and a significant percentage of bare soils (13% in each quadrant). In the south-eastern areas (quadrant 2), corn crops covered up to 20.5% of the surface, followed by cereal crops (16.2%). Quadrant 3 (south-west) was dominated by pastures and meadows (19.4%), along with corn (16%) and cereal (10%) crops. With regard to the cereals (table A2), the predominant crop was wheat (over 57% of cereal covered the area) for all quadrants with the exception of quadrant 4, in which rye was the prevailing crop (55% of the cropland). Conversely, Valladolid had a more homogeneous land use, and the territory was covered mainly by cereal crops (34.8% of the total area), especially in the northern, north-eastern and north-western territories. In the southern areas, a large area was covered by conifers (16.2% of the total grid), representing 28.7% of quadrant 2 and 23.8% of quadrant 3. Quadrant 1 also had a small area that was covered by pastures (11.2%), while in quadrant 3, 9.3% of the surface was covered by vineyards. The most common cereals in Valladolid were barley and wheat, with a very similar percentage of coverage.

Fig. 5 shows the behaviour of *Alternaria* spore concentrations depending on wind speed and direction. The bivariate polar plot in León (Fig. 5A) showed the presence of medium-low concentrations (20–30 spores m^{-3}) with a low wind speed from all directions. However, the south-west directions had similar concentrations with higher wind speeds (≈4 ms^{-1}). This behaviour can also be observed in the conditional probability function (CPF) plot, which showed low probability values for concentrations above the 90th percentile (equivalent to 53 spores/m^3) from all directions, although in this case, the south-western directions did not obtain greater probability values.

The highest concentrations in Valladolid (>80 spores/m^3) were detected from the north and north-west at wind speeds of between 1 and 3 m s^{-1} (Fig. 5A). Winds coming from the north-west similarly had a wide range of concentrations, always greater than 40 spores at different wind speeds, but never faster than 5 m s^{-1}. A low-medium concentration (40–50 spores/m^3) of between 2 and 3 m s^{-1} was also recorded for southern winds. Lower concentrations were recorded from the north-east, east and south-east, and the lowest concentrations occurred when the wind came from the south-west at speeds of 4–5 m s^{-1}. Both the CPF and the polar plot for Valladolid showed that the highest concentrations were associated with winds coming from north and north-western areas. However, the probability values of CPF were not high.

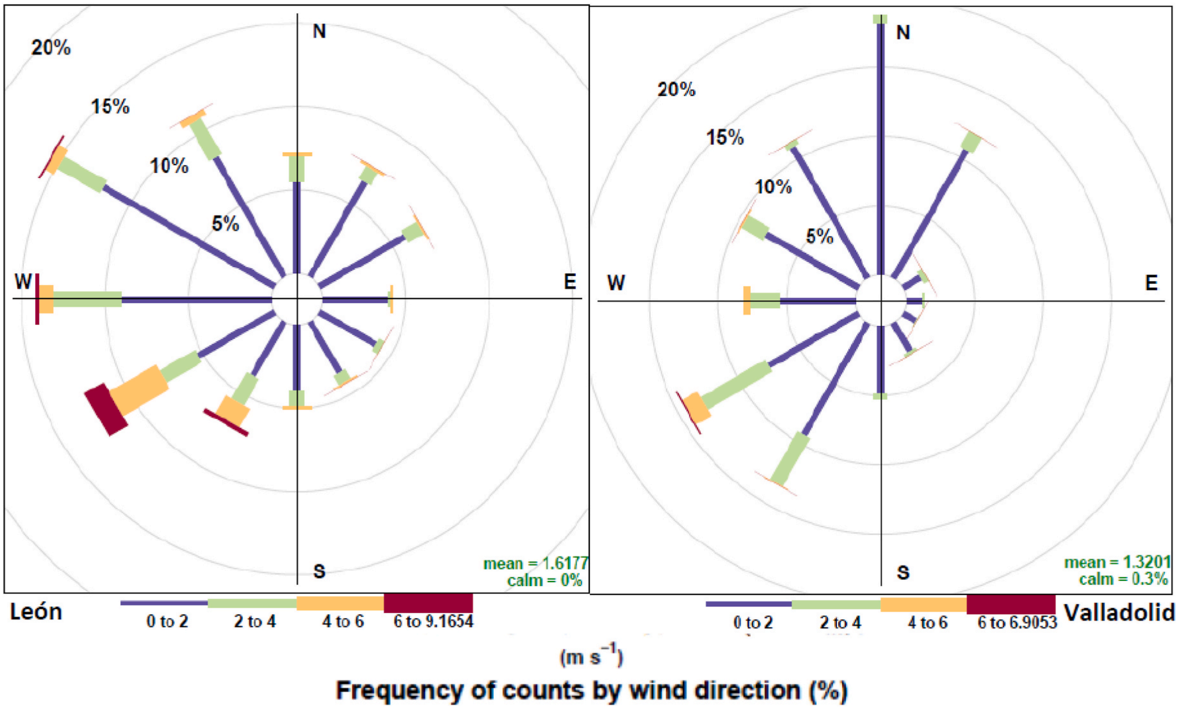


Fig. 3. Wind roses for wind speed/direction frequencies throughout the study period (2016–2020) in León (left) and Valladolid (right). Wind speed is represented by intervals in each plot, and the grey rings show the percentage (%) of frequency.

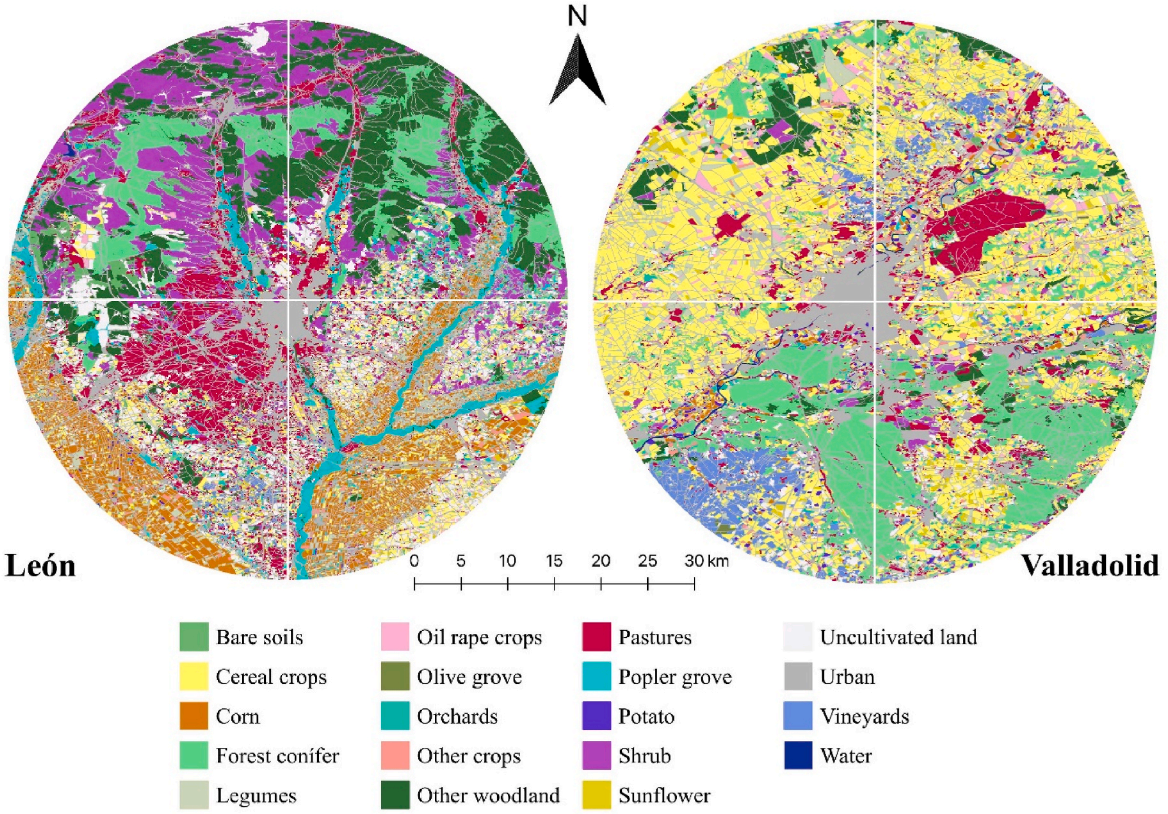


Fig. 4. Land cover in areas surrounding the traps (radius of 30 km) in León (left) and Valladolid (right).

The CPF also represents other possible emission sources from the west and southwest, but the probability value was very low.

Since León: I) had rare days with high spore concentrations (≥100 spores/m^3); II) did not showed a clear pattern of peak days throughout the sampling period, and III) presented many potential *Alternaria* emission sources surrounding the monitoring station, air mass back-

Table 2
Distribution of air mass trajectory origin and number of hours of air mass within 60 km of sampling point in León during risk days (≥100 sporesm^{-3}) and low concentration days. Results based on Fig. 6 and A.1.

Sector	Number of risk days (% total – Mean hours)	Number of low concentration days (% total – Mean hours)
NW	1 (2.7% - 12.9 h)	9 (27.3% - 4.2 h)
NE	7 (18.9% - 9.7 h)	11 (33.3% - 12.2 h)
SW	12 (32.4% - 8.0 h)	5 (15.1% - 9.2 h)
SE	17 (45.9% - 6.4 h)	8 (24.2% –6.0 h)

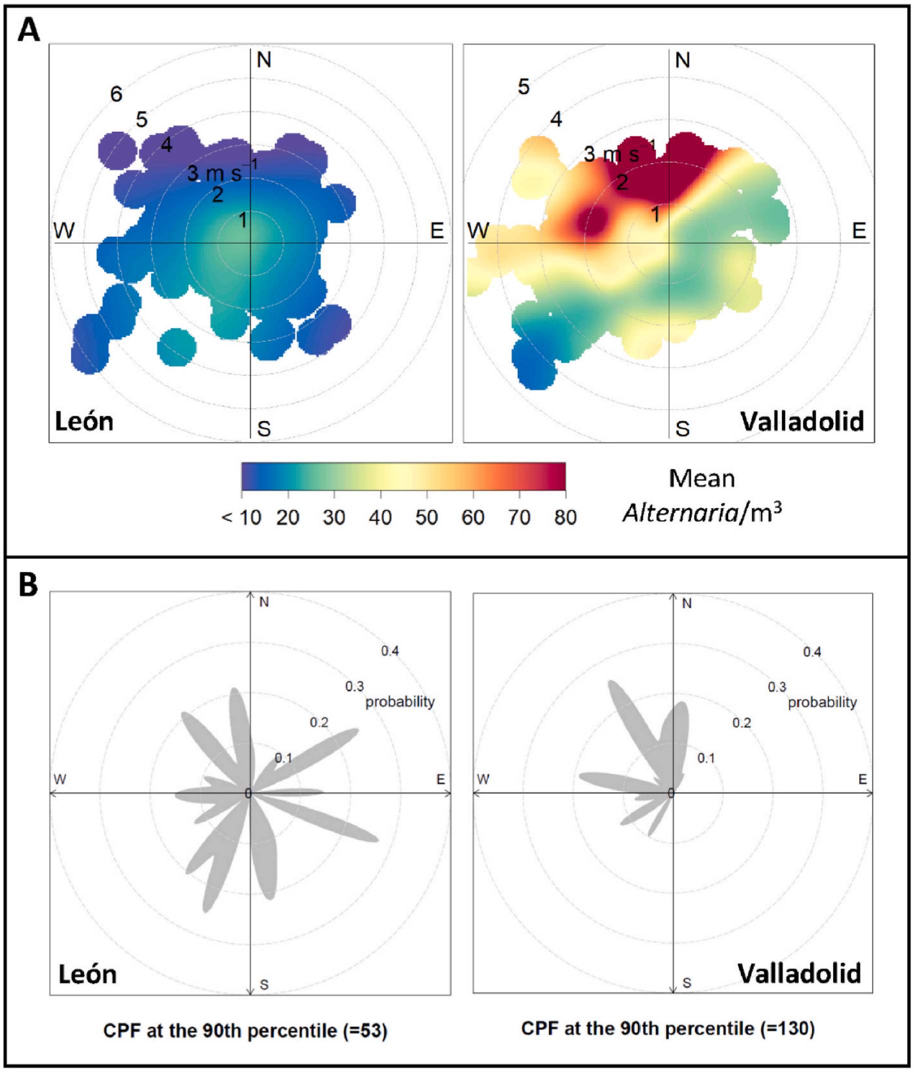
trajectories during risk days were calculated (Fig. 6). Trajectories were also obtained on randomly selected days with low concentrations (Figure A1) throughout the sampling period.

Table 2 shows the number of hours of air masses within 60 km of the monitoring station for each geographical sector. With regard to days with concentrations of ≥100 spores/m^3, air masses came mainly from the south (SW and SE) on more than 75% of the days, coinciding with the presence of pastures and agricultural areas (Fig. 4). However, on days with a low concentration, the air masses came mainly from the north (NW and NE) in more than 60% of cases. As an example, the origin was the NW on only one day (2.7% of cases) during days with concentrations of ≥100 spores/m^3. This contrasts with 27.3% of the cases for which the origin was NW during days with a low concentration.

With regard to the time the air masses remained at 60 km around the sampling point, there were no differences between days with concentrations of ≥100 spores/m^3 and days with a low concentration.

4. Discussion

The presence of *Alternaria* airborne conidia in the atmosphere is closely linked with meteorological factors, with the temperature being one of the most important parameters (O'Connor et al., 2014). The two cities analysed in this manuscript had a single sporulation period, which took place during the summer months, especially in July. This behaviour has already been observed in other northern Spanish locations (Aguilera et al., 2015; De Linares et al., 2010; Rodríguez-Rajo et al., 2005), and in many European cities (Grinn-Gofroń et al., 2019; Kasprzyk et al., 2015; Skjøth et al., 2016). According to Picornell et al. (2022), one spore season per year occurs in territories whose summer and autumn temperatures are below 32.6 °C and 11.4 °C, respectively. This corresponds with the temperatures that characterise the areas selected, with the maximum temperature of the warmest month being 27.8 °C in León and 29.4 °C in Valladolid (Rodríguez-Fernández et al., 2022). Higher



CPF at the 90th percentile (=53)

CPF at the 90th percentile (=130)

Fig. 5. A) Bivariate Polar plot for daily *Alternaria* spore concentration during MSS in accordance with wind speed and direction. Rings represent the wind speed in ms^{-1} and the colour scale shows the daily mean spore concentrations (*Alternaria*/m^3) for the period studied (2016–2020). B) Conditional probability function plot (CPF) of *Alternaria* concentrations >90th percentile.

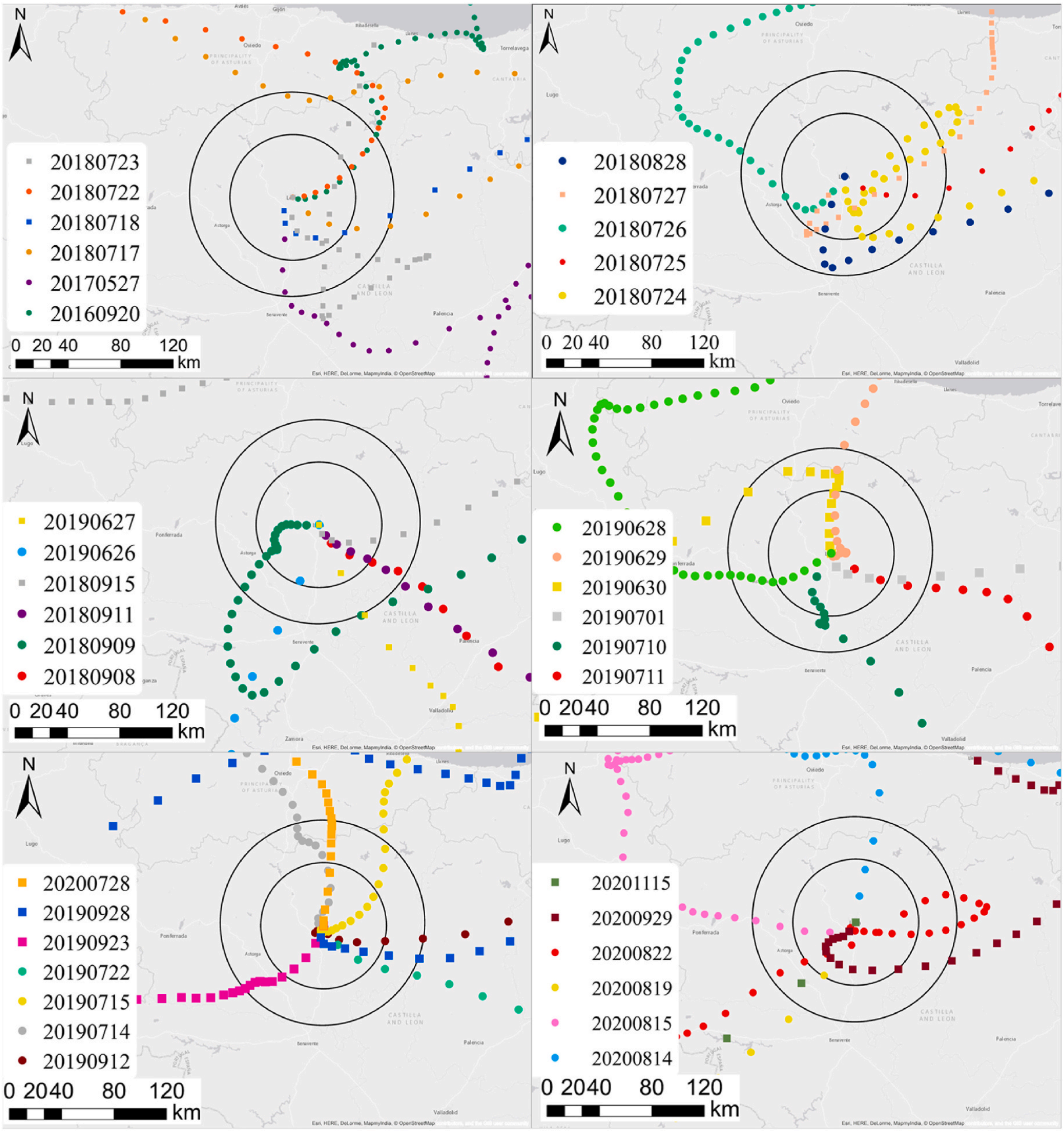


Fig. 6. 48 h back trajectories (200 m a.g.l.) arriving in León during peak days throughout sampling period. Each point is 1 h. Circles indicate 30 and 60 km around sampling point and the date is indicated in the format "yyyymmdd".

temperatures induce the split of the MSS into two seasons (spring and autumn), as usually occurs in southern Spanish locations (Maya-Manzano et al., 2012; Recio et al., 2011; Sabariego et al., 2012) owing to a decrease in spore fungal production, particularly for *Alternaria* spp. (Damialis et al., 2015).

The two areas selected, which are relatively few kilometres apart (140 km), were very different in terms of spore concentration, onset and length of the MSS. In all the years analysed, Valladolid had almost twice the concentration of León. These differences in spore concentrations may be partly owing to the variety of climate types in Castilla y León (ITACYLAEMET, 2013). The climate type in Valladolid is defined by the presence of hot dry summers, which favours the dispersion of *Alternaria* conidia, with the greatest concentrations occurring during these months, as our results and previous studies show (Sánchez-Reyes et al., 2016). However, the city of León is located at a northern latitude, and is defined by another climate type in which summer temperatures are lower while the precipitations are slightly higher. The differences between these spore concentrations in relatively close areas have been reported in other locations characterised by temperate and continental climates in which there is a wide-ranging annual temperature (Grinn-Gofroń et al., 2020). Moreover, this variability in spore concentrations may also be influenced by the altitude, since greater *Alternaria* conidia concentrations are commonly recorded in flat areas (Tomassetti et al., 2012). These findings coincide with our results, because the highest ASIn values were registered in Valladolid, which is located on a plateau over 700 m a.s.l., while León is located at a higher altitude (840 m a.s.l) and near the mountains. The biogeographical location of the areas analysed may also explain the differences as regards the start and duration of the MSS. Valladolid had an earlier onset and a greater number of days of MSS than León. This pattern was also observed in the study conducted in Castilla y León on the basis of the historical pollen database, in which two clusters were defined according to pollen season, with northern locations having a later and shorter season (Rodríguez-Fernández et al., 2022). This south-north gradient has also been reported in other studies, with northern and higher altitudinal locations being those that have later starts and a shorter duration of MSS (Grinn-Gofroń et al., 2019; Picornell et al., 2022; Skjøth et al., 2016).

Furthermore, there were notable annual differences between the ASIn values recorded in each city during the study period. This annual variation in the spore load on a specific site is influenced by numerous factors (Aira et al., 2013). In our study, the lowest ASIn values were recorded in 2016 in both cities. This was probably owing to the very low precipitation registered during the summer months, which were 17.9 mm in León and 11.9 mm in Valladolid. These precipitation values were well below the normal values for that period in the study area (Rodríguez-Fernández et al., 2022). Precipitation is another important meteorological parameter, which has a great impact on the annual variation in airborne spore load. Although this parameter is usually related to a negative impact owing to the scavenging effect on airborne particles (Blanco-Alegre et al., 2021), its pattern distribution throughout the year is of great importance because it is necessary for mould growth and spore production (Aira et al., 2008; Damialis et al., 2015; De Linares et al., 2010; Straatsma et al., 2001). However, according to Skjøth et al. (2016), this hypothesis cannot be fully supported because the number of years analysed in this manuscript are not sufficient to study the annual variability on a specific site.

The spatial variation in spore load found in the region cannot be explained entirely by climate and biogeographical factors, since previous studies have demonstrated the influence of potential emission sources and favorable conditions for conidia transport (Fernández-Rodríguez et al., 2015; Sadyś et al., 2014; Skjøth et al., 2016). It was for this reason that Grinn-Gofroń et al. (2020) stressed the importance of combining land cover studies with wind direction and speed analysis in order to attain better knowledge of the possible arrival of air masses with a high concentration of spores.

The vegetation in León is heterogeneous within 30 km of the radius analysed, signifying that there may be numerous emission sources. The prevailing winds in this city come from areas in which woodlands, shrublands and pastures cover a great area, and these kinds of vegetation are catalogued as important contributors of airborne spores (Humphrey et al., 2000; Redondo et al., 2020). However, as the polar plot and CPF plot showed, León is more affected by sources that are located closer to the monitoring station than forest and shrubland. The land cover map showed that pastures were the dominant vegetation in areas close to the spore trap. This relationship is consistent, because many species that grow in these environments are host plants of *Alternaria*, among them taxa belonging to Poaceae family, which act as emission sources (Frużyńska-Jóźwiak and Andrzejak, 2007; Sadyś et al., 2015; Schafer and Kotanen, 2004; Zahid et al., 2002). These findings support the hypothesis of Sadyś et al. (2015), that the contribution made by pastures and meadows to the *Alternaria* spore load cannot be underestimated. Moreover, the similar patterns found for Poaceae pollen (Oduber et al., 2019) and *Alternaria* spores in the city may be helpful as regards establishing local pathways of *Alternaria* dispersion using pollen data. Non-native ragweed pollen data have also been used to identify the long distance transportation of *Alternaria* spores (Grewling et al., 2019). These results could, therefore, be very useful in places in which *Alternaria* airborne spores are not monitored.

Most of the area in Valladolid was, in contrast, occupied by agricultural land, with wheat and barley being the most abundant cereal crops. This explains the high ASIn values recorded in this territory, since positive correlations between *Alternaria* concentration and the surface put to agricultural use have been found by other authors (Grewling et al., 2022; Grinn-Gofroń et al., 2020; Olsen et al., 2020). Moreover, the dominant air flows came from northerly directions that passed over large areas covered by cereal crops. This process favours spore dispersion from the major sources to the city, thus increasing the presence of *Alternaria* airborne conidia, as Grewling et al. (2022) also observed in Poland. However, prevailing winds from the southwest had a lower impact on the spore load, as can be seen in the polar plot and the CPF plot. These areas had different land uses in which pine groves and vineyards occupied more territory, and they do not appear to be significant sources of *Alternaria*. In contrast, Grinn-Gofroń et al. (2020) observed that coniferous forests were greater contributors of fungal spores.

With regard to the important role played by wind speed in *Alternaria* dispersal, both of the cities analysed had high spore counts with speeds ranging from <1 m s^{-1} to 5 m s^{-1}. This relationship has been observed to a much lesser extent in previous studies using correlation analysis, although they were unable to establish a wind speed range (Fernández-Rodríguez et al., 2015; Filali Ben Sidel et al., 2015; Grinn-Gofroń and Bosiacka, 2015; Sabariego et al., 2012; Sánchez-Reyes et al., 2016). Other studies based on back-trajectory analyses have provided more accurate results. For example, Skjøth et al. (2012) observed that the most effective wind velocities for spore dispersion in Copenhagen ranged from 1 to 6 m s^{-1}, while in Worcester, the elevated concentrations were recorded when wind speed varied between 3 and 4 m s^{-1} (Sadyś et al., 2015). These results are similar to those obtained in this study using a bivariate polar plot, signifying that this methodology could be a good tool with which to establish relationships between airborne spores and winds parameters (Grinn-Gofroń et al., 2020). These low-medium wind speed values point to the influence of local sources in airborne spore concentration. However, occasional events of medium-distance transport can also occur with low-medium wind speeds when there is a high wind persistence (Damialis et al., 2005).

The great variation in spatial spores observed in this manuscript is a result of all the different aforementioned parameters. Nevertheless, agricultural management also has a great impact on concentrations of *Alternaria* because it results in huge amounts of conidia being released into the atmosphere (Friesen et al., 2001; Olsen et al., 2019; Skjøth et al., 2012). The maximum concentration days in Valladolid occurred on similar dates during the years analysed. These dates corresponded to the cereal harvest period, when an important part of the surface had already been harvested (BOCYL, 2010). This agricultural process, together with the origin of prevailing winds that is described above, explains the high concentration of *Alternaria* recorded in this city. This study, therefore, supports the huge impact of agricultural management on *Alternaria* airborne concentrations observed by other authors (Apangu et al., 2020; Sadyś et al., 2015; Skjøth et al., 2012). Moreover, these findings coincide with the seasonal distribution of allergic symptoms triggered by fungi described in the city (Armentia et al., 2019), and these results will consequently make it possible to predict the period with the highest risk for allergic population sensitised to *Alternaria*.

The contribution of agricultural activities in León was, in contrast, not as evident as in Valladolid. The few days with a concentration of $\geq$100 spores m^{-3} did not have a pattern in the study period, and could occur at any time throughout the year. This behaviour does not allow the establishment of specific risk period for allergic people. Nonetheless, it is important to note that the threshold value for evoking respiratory symptomatology in sensitised individuals chosen in this study could be overestimated, as has been described for other aeroallergen (Steckling-Muschack et al., 2021), which is a limitation of the study. The analysis of air masses during risk days revealed the importance of taking this parameter into account in *Alternaria* forecasting models. The results showed that days with a high concentration in León were recorded when air masses came from southerly directions, coinciding with the areas covered by croplands. These wind directions are infrequent in León, which explains the temporal variation of peak days among years, along with the low concentration of *Alternaria* recorded in the city. Finally, our results showed that in León, the origin is more important for the *Alternaria* concentration than the time that the air mass stays around the monitoring station.

5. Conclusions

The two locations selected in this study show the great differences that may exist as regards the presence of *Alternaria* airborne spores in nearby areas. This highlights the importance of conducting aerobiological spore studies at a local level. Our results suggest that the MSS is strongly influenced by bioclimatic and orographic characteristics, and there is a pattern for the onset and length of the MSS based on the variability of the territories on these factors. Furthermore, the differences between the two territories as regards *Alternaria* airborne spore concentrations are owing mainly to the surface occupied by the emission sources and the arrival of air masses from these areas. The major *Alternaria* emission sources were cereal lands, followed by grasslands, which have a lower impact. However, forests, pine plantations, shrublands and vineyards do not seem to contribute to the *Alternaria* spore load. The high concentrations recorded in Valladolid are owing to the prevailing winds with low speeds that favour a continuous transportation of spores from the cereal crops to the city. The strong impact that the harvest season has on the amounts of spore in this city can also be observed, and this is the period of greatest risk for the *Alternaria* allergic population. However, the highest concentrations in León were not linked to the prevailing winds, but rather to winds with infrequent directions. All of the above stresses the importance of knowing the spore airborne transport routes for the prediction of risk periods.

Author contributions

Alberto Rodríguez-Fernández: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing-Original draft. Carlos Blanco-Alegre: Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Writing-Review & editing. Ana María Vega-Maray: Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing-Review & editing. Rosa María Valencia-Barrera: Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing-Review & editing. Tibor Molnar: Formal analysis, Resources, Software, Visualization. Delia Fernández-González: Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing-Review & Editing.

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 are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.117414.

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