



Potential sources of airborne *Alternaria* spp. spores in South-west Spain



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HIGHLIGHTS

- Counts of *Alternaria* spores and analysis of habitats
- Study of air mass transport and meteorological data
- Analysis of back-trajectories of *Alternaria* in Spain

ARTICLE INFO

Article history:

Received 6 March 2015

Received in revised form 19 May 2015

Accepted 7 June 2015

Available online xxxx

Editor: D. Barcelo

Keywords:

Aerobiology

Alternaria habitats

HYSPLIT

Air mass transport

Back-trajectory analysis

ABSTRACT

Fungi belonging to the genus of *Alternaria* are recognised as being significant plant pathogens, and *Alternaria* allergens are one of the most important causes of respiratory allergic diseases in Europe. This study aims to provide a detailed and original analysis of *Alternaria* transport dynamics in Badajoz, SW Spain. This was achieved by examining daily mean and hourly observations of airborne *Alternaria* spores recorded during days with high airborne concentrations of *Alternaria* spores ($>100 \text{ s m}^{-3}$) from 2009 to 2011, as well as four inventory maps of major *Alternaria* habitats, the overall synoptic weather situation and analysis of air mass transport using Hybrid Single Particle Lagrangian Integrated Trajectory model and geographic information systems. Land use calculated within a radius of 100 km from Badajoz shows that crops and grasslands are potentially the most important local sources of airborne *Alternaria* spores recorded at the site. The results of back trajectory analysis show that, during the examined four episodes, the two main directions where *Alternaria* source areas were located were: (1) SW–W; and (2) NW–NE. Regional scale and long distance transport could therefore supplement the airborne catch recorded at Badajoz with *Alternaria* conidia originating from sources such as crops and orchards situated in other parts of the Iberian Peninsula.

Published by Elsevier B.V.

1. Introduction

Fungi belonging to the genus of *Alternaria* are recognised as being significant plant pathogens. The majority of *Alternaria* species are secondary invaders. They are rarely considered to be virulent pathogens but can still produce considerable economic losses worldwide (Rotem, 1994). Predictions of airborne *Alternaria* spores can be used to optimize fungicide applications in cereals (Aira et al., 2013) and aid in the fight against phytopathogens in crops such as cotton (Bashan et al., 1991) and potato (Escuredo et al., 2011). In addition, *Alternaria* spp. (hereafter *Alternaria*) allergens are one of the most important causes of respiratory

allergic diseases in Europe (D'Amato et al., 1997) and globally (Denning et al., 2006), with the standardised sensitization rate to *Alternaria* allergens in Europe being reported as 8.9% (Heinzerling et al., 2009). It has been shown that patients sensitized to *Alternaria alternata* were more frequently affected by asthma compared to the patients who were not sensitized to this aeroallergen (Bartra et al., 2009). Studies on the paediatric population have also shown that, in children, the prevalence of *A. alternata* sensitization is significantly higher (Bartra et al., 2009), *Alternaria* spores may be an important cause of allergic rhinoconjunctivitis (Andersson et al., 2003) and airborne fungal spores, including *Alternaria*, may provoke or exacerbate asthma attacks (Atkinson et al., 2006). Models of spatial and temporal variations in airborne *Alternaria* fungal spore concentrations may therefore also serve as an early warning to sensitized allergy-sufferers (Aira et al., 2013).

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The conidia of *Alternaria* species may be dispersed by rain splash, but the main dispersal mechanism is passively via air currents (Aylor, 1990; McCartney et al., 1993). A number of studies have examined the long distance transport (LDT) of airborne pollen grains and attempted to identify their sources, e.g.: *Ambrosia* spp. (Stach et al., 2007; Smith et al., 2008; Sikoparija et al., 2009, 2013), *Betula* spp. (Skjøth et al., 2007, 2009), *Quercus* spp. (Hernández-Ceballos et al., 2011b), *Olea* spp. (Hernández-Ceballos et al., 2011a; Fernández-Rodríguez et al., 2014), and Poaceae (Smith et al., 2005). On the other hand, similar studies for airborne fungal spores are less common, e.g.: *Ganoderma* spp. in the UK (Sadyś et al., 2014a) and *Alternaria* in the UK and Denmark (Skjøth et al., 2012; Sadyś et al., 2014b).

The present study aims to connect atmospheric concentrations of *Alternaria* spores recorded in Badajoz, Spain, to different types of agricultural land in the surrounding areas by examining air mass transport. This will be achieved by using daily mean and hourly observations of airborne *Alternaria* spores recorded at Badajoz during days with high airborne concentrations of *Alternaria* spores ($>100 \text{ s m}^{-3}$) from 2009 to 2011, as well as four inventory maps of major *Alternaria* habitats, the overall synoptic weather situation and analysis of air mass transport using HYSPLIT and geographic information systems. Some general aerobiological studies on airborne *Alternaria* spores have previously been published with data from the study area (Maya-Manzano et al., 2012; Aira et al., 2013), but this is the first detailed analysis of atmospheric transport of *Alternaria* spores in Badajoz. The results of this study are valuable because they can be used for identifying and mapping sources of airborne *Alternaria* spores in the region. This information can be used to prepare source inventories for dispersion modelling, in a similar way that sources of *Ambrosia* pollen were first identified (Stach et al., 2007; Sikoparija et al., 2009, 2013; Smith et al., 2009) and later mapped (Skjøth et al., 2010; Thibaudon et al., 2014; Karrer et al., 2015). The output from such models can be used for several applications, including the production of bulletins aimed at health care professionals and the allergic population, or to provide information about the potential spread of plant pathogens in agriculture, or both.

2. Material and methods

2.1. Sampling site and inventory of potential *Alternaria* spore sources

Badajoz is the largest city (1470 km^2) in the agricultural province of Extremadura region, in South-West Spain, with a population of 151,565 (NSI, 2015). Badajoz is located 184 m above sea level and only about 2 km away from the border with Portugal (Fig. 1a). Around the city of Badajoz there are irrigated crops (e.g. fruit, corn, tomato, rice), olive trees, more distant holm-oak trees and extensive grazing (Fig. 1b).

Major *Alternaria* habitats were identified using the Corine Land Cover 2000 (CLC 2000) dataset for the studied area (EC. European Commission, 2005). Seinfeld and Pandis (2006) proposed the following classification for the dispersal of aerosols in the air: (a) micro-scale (up to 100 m), (b) urban-local scale (100 m–10 km), (c) regional or meso-scale (10 km–1000 km), and (d) synoptic or global scale (of more than 1000 km). These classifications are now commonly applied in the aerosol sciences. Although, a more detailed classification proposed by Orlanski (1975) separates the mesoscale into 2–20 km, 20–200 km, and 200–1000 km. In this study, we examine regional/meso-scale atmospheric transport. The radius of 100 km distance from Badajoz was chosen following Avolio et al. (2008) who suggested that (for pollen sized particles) the typical transport distance is in the range of 30–100 km. The inventory maps were gridded using the CLC 2000 projection by aggregating the grid cells to $5 \text{ km} \times 5 \text{ km}$ using similar procedures as Skjøth et al. (2012) and Sadyś et al. (2014b). This procedure enables an easy comparison of the density of habitats at regional and national scales described in previously published studies.

Alternaria species frequently attack cultivated cereals (Table 1). Four habitats where *Alternaria* conidia are known to be present in this area were selected for this study: (1) crops; (2) grasslands and pastures; (3) orchards; (4) vineyards (Table 1). Maps representing each of these four habitats were produced. Firstly, a habitat map showing the distribution of crops in Spain was prepared upon a joint extraction of the: (a) non-irrigated arable land (code 211), (b) permanently irrigated

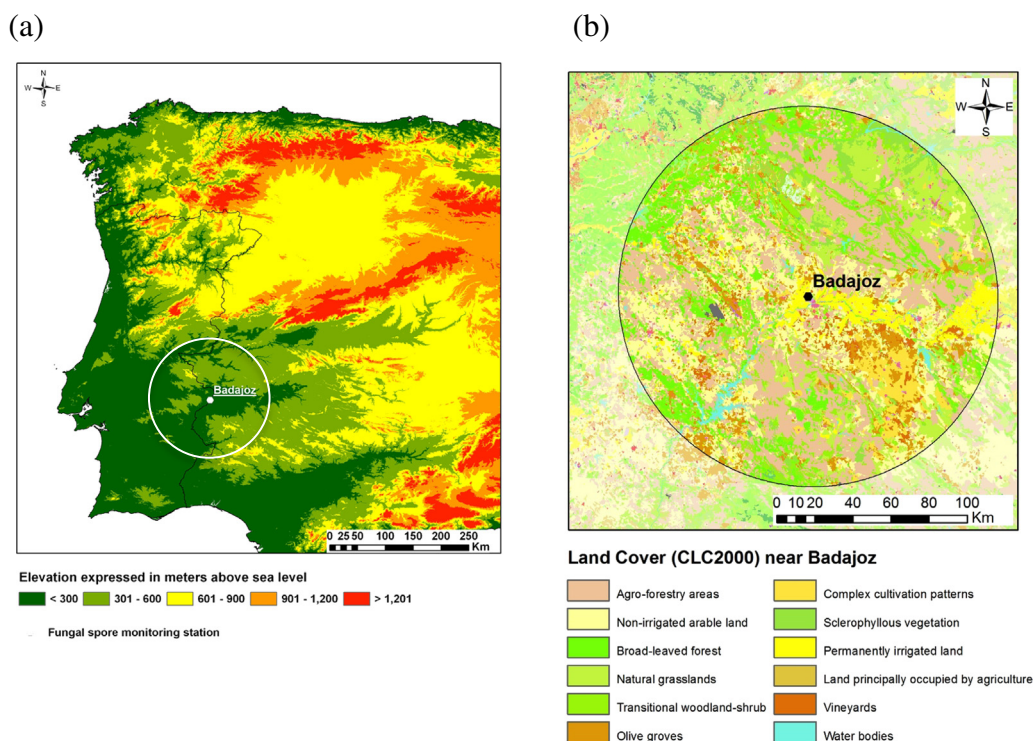


Fig. 1. Site of the study in Badajoz, Spain: (a) hypsometric map; (b) land use within a 100 km radius of the city.

Table 1
Biodiversity of *Alternaria* species detected in examined habitats.

Habitat	Species	Hosts	Studies
Crops (cereal)	<i>Alternaria alternata</i>	Barley	Müller and Korn (2013)
	<i>Alternaria humicola</i>	Oat	Nicolaisen et al. (2014)
	<i>Alternaria infectoria</i>	Wheat	Uddin and Chakraverty (1996)
	<i>Alternaria tenuissima</i>		
	<i>Alternaria triticina</i>		
Crops (other)	<i>Alternaria alternata</i>	Bean	Allen et al. (1983)
	<i>Alternaria arborescens</i>	Blueberry	Boedo et al. (2012)
	<i>Alternaria brassicicola</i>	Cabbage	Chaerani and Voorrips (2006)
	<i>Alternaria cucumerina</i>	Carrot	Escuredo et al. (2011)
	<i>Alternaria dauci</i>	Leek	Greco et al. (2012)
	<i>Alternaria helianthi</i>	Linseed	Meredith (1966)
	<i>Alternaria linicola</i>	Onion	Modesto Olanya et al. (2009)
	<i>Alternaria porri</i>	Potato	Schenk (1968)
	<i>Alternaria raphani</i>	Sunflower	
	<i>Alternaria solani</i>	Tomato	
	<i>Alternaria tenuissima</i>	Watermelon	
Pastures	<i>Alternaria alternata</i>	Grasses	Frużyńska-Józwiak and Andrzejak (2007)
	<i>Alternaria nobilis</i>	Knapweed	Garibaldi et al. (2013)
	<i>Alternaria tenuissima</i>	Soapwort	Müller (1992)
		White clover	Zahid et al. (2002)
Orchards	<i>Alternaria alternata</i>	Almond	Biton et al. (2014)
	<i>Alternaria arborescens</i>	Apples	De Linares et al. (2010)
	<i>Alternaria longipes</i>	Clementine	De Souza et al. (2009)
	<i>Alternaria mali</i>	Lemon	Harteveld et al. (2014)
	<i>Alternaria tenuissima</i>	Olive	Luo et al. (2007)
		Persimmonia fruit	Moral et al. (2008)
		Pistachio	Ortuño et al. (2008)
		Pomelo	Rotondo et al. (2012)
		Satsuma	Vicent et al. (2009)
		Tangarine	
		Tangor	
Vineyards	<i>Alternaria alternata</i>	Grape	Eltem et al. (2009)
	<i>Alternaria arborescens</i>		Lorenzini and Zapparoli (2014)
	<i>Alternaria tenuissima</i>		Ostrý et al. (2007)

arable land (code 212), (c) rice fields (code 213), (d) olive groves (code 223), and (e) agro-forestry areas (code 244). In this study, crops are understood as being cereals (oat, wheat and barley) as well as economically important plants such as potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), and onion (*Allium cepa*). Secondly, a habitat map that only contained areas occupied by pastures (code 231) and grasslands (code 321) was prepared. Finally, habitat maps for orchards (code 222) and vineyards (code 221) were also constructed.

A hypsometric map with a spatial resolution of 90 m was produced using the Digital Elevation Model (DEM) available at CGIAR-CSI SRTM Database (<http://srtm.csi.cgiar.org>).

2.2. Climate and meteorological data

Badajoz has a Mediterranean climate affected by a degree of continentality, with autumn and winter maximum precipitation and a dry period in summer when the highest temperatures are recorded (Tormo-Molina et al., 2010). Based on the meteorological data recorded at Badajoz Airport (38° 53' N, 6° 49' W), annual average rainfall is 463 mm and annual average temperature is 16.6 °C (AEMET, 2011). The orography of the region is flat (Fig. 1a), with prevailing winds blowing from the West (Silva-Palacios et al., 2007).

Hourly meteorological data for Badajoz were provided by the meteorological station located at the experimental area at the experimental garden that belongs to the Faculty of Science, at the University of Extremadura, situated 16 m away from the spore-monitoring site. The following meteorological parameters were examined in this study: mean air temperature [°C]; mean ground temperature [°C]; solar radiation [W m^{-2}], relative humidity [%], and wind speed [m s^{-1}].

The overall synoptic weather situation was investigated using weather maps provided by the UK Met Office, as a part of the agreement with the National Centres for Environmental Prediction (NCEP). The

weather maps represented the synoptic situation at 00:00 UTC each day.

2.3. *Alternaria* spore data

The spore-monitoring site in Badajoz is situated at 16 m above ground level, on the terrace of the Faculty of Science building, at the University of Extremadura (38° 52'00"N, 7° 00'10"W). Daily average and diurnal (hourly) *Alternaria* spore concentrations from 2009 to 2011 were obtained using volumetric spore trap of the Hirst design (Hirst, 1952). Air is sucked into the trap at a rate of 10 l/min through a 2 mm × 14 mm orifice. Behind the orifice the air flows over a rotating drum that moves past the inlet at 2 mm/h and is covered with an adhesive coating, transparent plastic tape. Particles in the air impact on the tape to give a time related sample (Emberlin, 2000). The sampler used in this study was located on an open terrace with the intake at 1.5 m from the floor. Standardised data management procedures were used, similar to those described by the Spanish Aerobiology Network (REA) (Galán et al., 2007). Slides were examined along two longitudinal transects, which equates to 7.7% of the slide examined. Daily average (00:00 to 23:59) *Alternaria* spore concentrations and diurnal variations (hourly concentrations) were expressed as the number of spores per cubic metre of air [s m^{-3}].

The *Alternaria* spore seasons were defined using the 90% method, whereby the season started when 5% of the total annual catch was achieved and ended when 95% was reached (Nilsson and Persson, 1981). The characteristics of *Alternaria* spore seasons were described by the start and end dates of the fungal spore season, the duration (in days), the date of the maximum daily average *Alternaria* spore concentration, the maximum daily average *Alternaria* spore concentration and the sum of daily average airborne *Alternaria* spore concentrations recorded in the season (Seasonal Spore Index – SSI). The mean diurnal (hourly) variation of all days with a daily average airborne *Alternaria*

spore concentration $>100 \text{ s m}^{-3}$ recorded during the season in Badajoz was examined. The threshold of $>100 \text{ s m}^{-3}$ was simply an arbitrary figure selected for removing days with low daily average values, whilst still allowing a representative size of the sampled days for analysis. Although it should be noted that the onset of allergenic symptoms in atopic subjects frequently occurs when airborne concentrations of *Alternaria* spores are equal to or greater than 100 s m^{-3} (Ballerio et al., 1984; Ricci et al., 1995).

2.4. Back-trajectory analysis and selection of episodes

The air mass transport above Badajoz was examined using clusters of the back trajectories computed by the HYSPLIT model (Draxler and Hess, 1998; Draxler and Rolph, 2014; Rolph, 2014) for all days with high airborne concentrations of *Alternaria* spores ($>100 \text{ s m}^{-3}$, $n = 86$), (Fig. S1). The weather data used for the trajectory calculation came from the Global Data Analysis System (GDAS) archives, which is maintained by the National Oceanic and Atmospheric Administration Air Resources Laboratory (NOAA ARL). Air mass trajectories were plotted 24 h back in time, with one-hour interval, at 500 m above ground level. Episodes with high atmospheric concentrations of *Alternaria* spores ($>100 \text{ s m}^{-3}$) and diurnal patterns that were distinctly different to the mean daily cycle were selected for further analysis with back-trajectory analysis following the methodology described in previous studies (Skjøth et al., 2009; Fernández-Rodríguez et al., 2014). From these, episodes deemed to be examples of different air mass movement were selected for detailed investigation using the following criteria: (i) constancy of the air mass direction throughout the entire episode, and (ii) the pattern of air mass distribution. The position of the air masses in relation to different *Alternaria* habitats can be found in the supplementary material (Figs. S2–S5).

Airborne concentrations of *Alternaria* spores are influenced by daytime meteorological conditions that increase spore release and dispersal (Rotem, 1994) and so the obtained trajectories were divided into two clusters: day (08:00–19:00) and night (20:00–07:00). This selection was based on known literature concerned with the circadian distribution of *Alternaria* conidia in the air (Meredith, 1963; Leach, 1975; Maya-Manzano et al., 2012), and supported by the analysis of hourly concentrations of airborne *Alternaria* spores (Section 2.5). Trajectories were visually presented using a time scale of 6 h interval, i.e.: 00:00–06:00, 07:00–12:00, 13:00–18:00, and 19:00–23:59. In addition, the frequency of day and night air mass directions for each selected episode was determined using the spatial resolution of $1^\circ \times 1^\circ$ as described by Skjøth et al. (2012). Finally, the time that the air masses spent over specific areas was calculated using tools available in ArcGIS (v. 10) software.

2.5. Statistical analysis

Spearman's rank test was used to examine the relationship between the occurrence of *Alternaria* spores in the air in Badajoz and meteorological parameters. This association was studied using hourly records. Spearman's test was performed for each selected episode individually, as well as for daytime (08:00–19:00) and nighttime (20:00–07:00) hours, and with 24 h and 48 h lag between recorded airborne spore concentrations and weather parameters. SPSS (v.15.0) was used to perform this association test at the significance level of $p < 0.05$.

Multivariate Regression Trees (MRTs) were produced to indicate the time when greater or lower concentrations of *Alternaria* conidia were observed in the air of Badajoz during the examined episodes. MRTs were computed using the R software (CRAN, v. i386 3.1.0) and Multivariate Partitioning (mvpart) package (De'ath, 2002). To stabilise the Cross Validated Relative Error (CVRE) two thousand cross validations were performed. The lowest possible CVRE was the criterion upon which MRT models were selected, following De'ath and Fabricius (De'ath and Fabricius, 2000) and Grinn-Gofroń and Strzelczak (2009).

3. Results

3.1. Inventory of potential *Alternaria* spore sources

Land use calculated within a radius of 100 km from Badajoz shows that the most important components of the landscape are: agro-forestry areas with holm oak trees mainly (26.61%), non-irrigated arable land (17.20%), broad-leaved forests mainly of holm-oak trees and in lesser extent cork-oak trees (10.54%) and natural grasslands (9.92%) (Fig. 1b and Table 2). These land use classes roughly correspond to the habitat maps for crops, pastures, and grasslands (Fig. 2 a–b). Vineyards located within a 100 km radius from Badajoz cover 1.57% of the total area, whilst orchards (fruit trees and berry plantations) only cover 0.35% (Table 2).

Crops on the Iberian Peninsula cover the majority of agricultural areas and are mainly cultivated in the regions of Castilla-León, Castilla-La Mancha, Aragón, and Andalucía (Fig. 2a). In contrast, grasslands are mainly located along the northern border of Spain, in Asturias, Cantabria, Basque Country, and Navarra (Fig. 2b). High densities of orchards were found in the regions of Andalucía and Extremadura, although orchards can also be found in the vicinity of the cities of Barcelona and Madrid (Fig. 2c). Vineyards are dominant in the regions of Valencia and Murcia, which are situated towards the eastern border of Spain adjacent to the waters of the Balearic Sea (Fig. 2d). Crops and orchards located in southern Portugal and grasslands in southern France are also possible sources of airborne *Alternaria* spores.

3.2. Temporal variations in airborne concentrations of *Alternaria* spores

Alternaria spores recorded in Badajoz varied annually (Table 3). The main *Alternaria* spore season occurred from the beginning of May to the end of October, with the most of *Alternaria* conidia observed in June and October. The duration of the *Alternaria* spore season ranged between

Table 2

A summary of the land use within a 100 km radius of Badajoz, Spain. Note that a small fraction of which constitutes the territory of Portugal.

Land use category	Area [ha]	Area [%]
Agro-forestry areas	835,968	26.61
Non-irrigated arable land	540,318	17.20
Broad-leaved forest	331,016	10.54
Natural grassland	311,646	9.92
Transitional woodland-shrub	234,260	7.46
Olive groves	184,466	5.87
Complex cultivation patterns	157,074	5.00
Sclerophyllous vegetation	147,220	4.69
Permanently irrigated land	137,720	4.38
Land principally occupied by agriculture, with significant areas of natural vegetation	52,042	1.66
Vineyards	49,247	1.57
Water bodies	38,330	1.22
Annual crops associated with permanent crops	21,294	0.68
Mixed forest	16,529	0.53
Rice fields	11,806	0.38
Discontinuous urban fabric	11,524	0.37
Fruit trees and berry plantations	11,104	0.35
Continuous urban fabric	9541	0.30
Water courses	7323	0.23
Coniferous forest	6843	0.22
Pastures	6652	0.21
Sparsely vegetated areas	6553	0.21
Burnt areas	4668	0.15
Industrial or commercial units	3090	0.10
Mineral extraction sites	2720	0.09
Moors and heathland	757	0.02
Construction sites	718	0.02
Airports	445	0.01
Sport and leisure facilities	345	0.01
Other	149	0.01

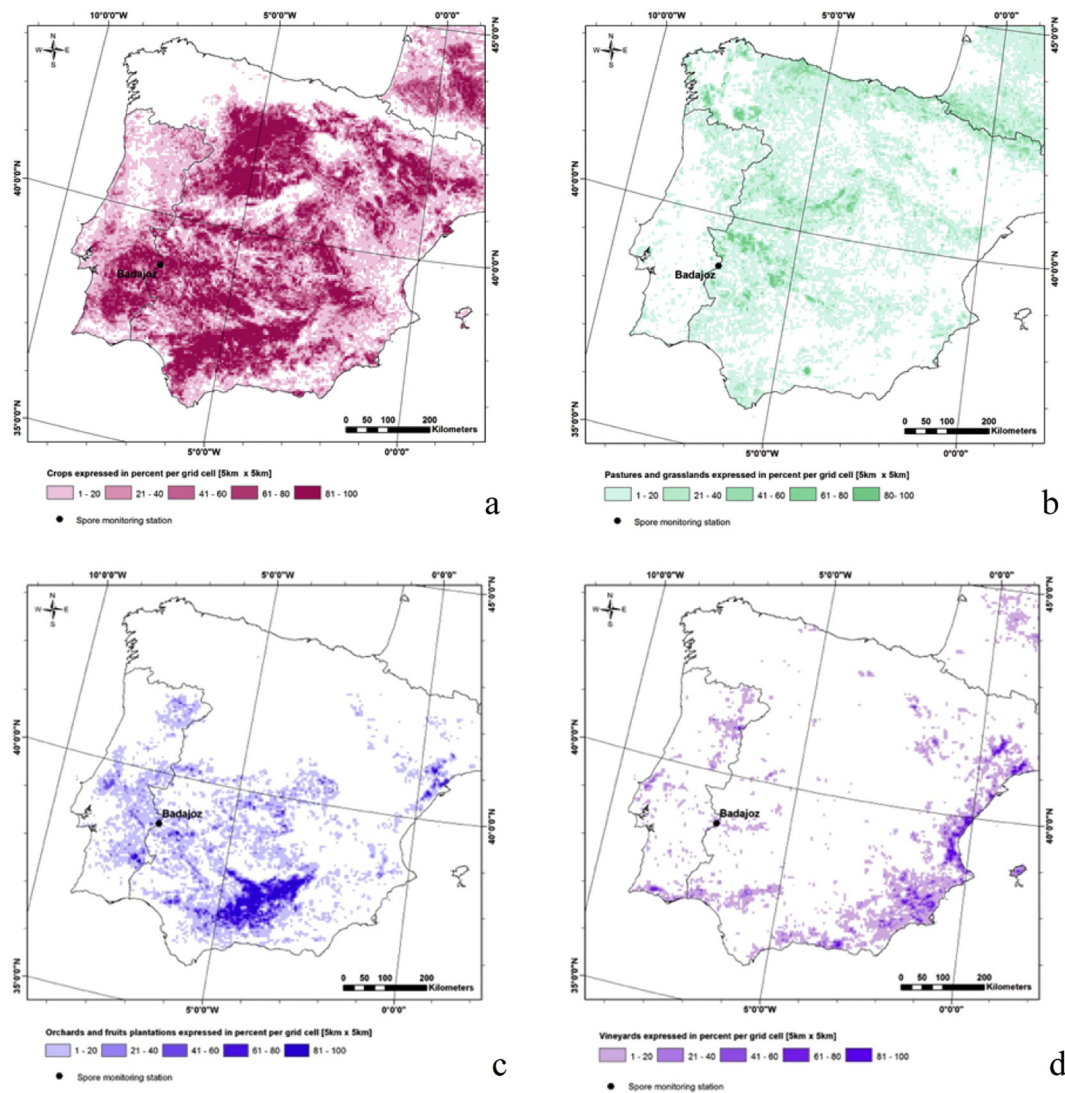


Fig. 2. Habitat maps of the four major *Alternaria* habitats in the studied area: (a) crops, (b) pastures and grasslands, (c) orchards, (d) vineyards.

161 and 190 days. The lowest and the highest SSI of *Alternaria* spores were recorded in 2009 and 2011, respectively. The highest daily average *Alternaria* spore concentration was 638 s m^{-3} recorded on 1 June 2011.

During the 3-year study, daily mean *Alternaria* spore concentrations exceeded 100 s m^{-3} on a total of 86 days. The mean diurnal cycle of these 86 days (Fig. 3) showed a peak in airborne *Alternaria* spores from about mid-morning (~10:00) to late afternoon (~16:00) with a midday maximum and nighttime minimum. A total of 19 episodes (each $\geq 48 \text{ h}$ long) with diurnal patterns that were distinctly different to the mean daily cycle were identified. Four of these episodes were selected for detailed investigation because they showed constancy in the direction taken by the air masses throughout the entire episode and the pattern of air mass distribution showed examples of different air

mass movement: (a) 29–30 Jun 2009, (b) 12–13 Oct 2009, (c) 14–15 Jun 2010, and (d) 24–25 May 2011. A detailed analysis of hourly concentrations of airborne *Alternaria* conidia from these four episodes selected showed a bimodal distribution (Fig. 4a–d). A regular increase in *Alternaria* spore concentrations was detected between 09:00 and 12:00. MRTs were used to examine the four episodes selected for further analysis using back trajectory calculations (Section 3.3). The MRT models showed that there were generally statistically significant differences in airborne concentrations of *Alternaria* spores recorded in Badajoz before and after dawn, with a greater contribution to the overall spore concentrations registered in the morning and mid-afternoon hours (Fig. 4a–c). A different result was obtained for the fourth examined episode (24–25 May 2011) when the majority of the spores were trapped between 19:00 and 02:00 (Fig. 1d) and there was a significant difference between airborne *Alternaria* spores recorded before and after 18:30.

The results of Spearman's rank correlation analysis showed that there were a number of statistically significant relationships between hourly concentrations of airborne *Alternaria* spores and meteorological data (Table 4). Mean air and ground temperatures frequently had a significant positive influence on airborne *Alternaria* spores. Whereas relative humidity often had a significant negative relationship with the amount of *Alternaria* spores recorded in the air. There tended to be significant positive correlations between airborne *Alternaria* spores and

Table 3
Characteristics of *Alternaria* spore seasons calculated using the 90% method in Badajoz (2009–2011).

Year	Start date	End date	Duration days	Peak day	Daily maximum	SSI
2009	1 May	6 Nov	190	17 Oct	215	8415
2010	3 May	28 Oct	179	14 Oct	389	8678
2011	28 Apr	5 Oct	161	1 Jun	638	13,696

*SSI — Seasonal Spore Index calculated as the sum of daily average spore counts recorded in the spore season.

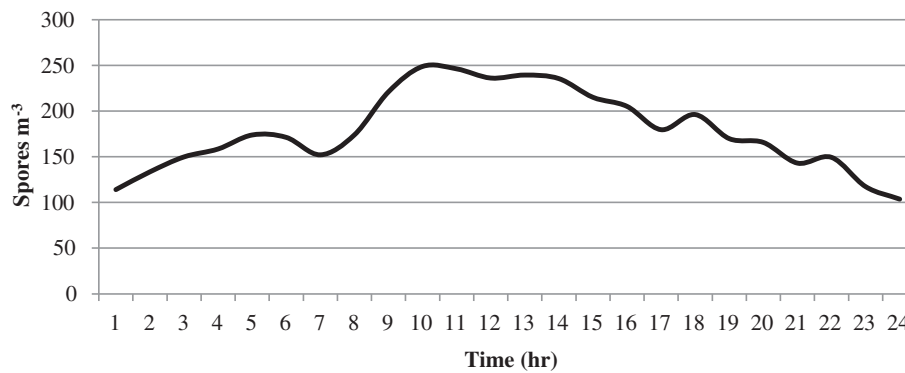


Fig. 3. Mean bi-hourly distribution of airborne *Alternaria* spores recorded in Badajoz for all days with high atmospheric concentrations of *Alternaria* spores ($>100 \text{ s m}^{-3}$) ($n = 86$).

solar radiation during the day, and significant negative correlations at night. However, during the fourth episode (24–25 May 2011) there were several significant positive correlations between airborne *Alternaria* spores and solar radiation recorded during nighttime (20:00–07:00). There were also a number of significant correlations between airborne *Alternaria* spores and wind speed, this relationship was generally positive but there was one significant negative correlation during nighttime in the second episode, 12–13 Oct 2009 (24 h lag).

3.3. Analysis of the air mass transport

3.3.1. Episode 1 (29–30 Jun 2009)

A sudden change in weather conditions in Spain was observed from 28 to 29 June 2009. A high-pressure system (1016 hPa) that brought clement weather to the Iberian Peninsula was displaced towards Turkey by a low-pressure system (983 hPa), which remained over Spanish territory for the following 72 h. Air masses during the examined episode mainly originated from the South-Southwest (181° – 225°) and Southwest-West (226° – 270°) directions during daytime, and from West-Northwest (271° – 315°) at night. No contribution was observed from the North-Northeast (0° – 45°) direction regardless of time of day (Fig. 5). The air masses spent the majority of the time over the Atlantic Ocean as they approached Badajoz: 67% during daytime hours and 73% at night. The air masses only spent a short time over land, the majority of which was spent over Portuguese rather than Spanish territory (Fig. 5). The results of back trajectory analysis (Fig. 6a–b) show that daytime and nighttime air masses approached Badajoz from a southwesterly direction, and so the major source areas were crops and orchards located in southern Portugal and the Extremadura region in Southwest Spain (Fig. 2).

3.3.2. Episode 2 (12–13 Oct 2009)

Conditions during the second episode were influenced by two different pressure systems; a low pressure system that formed on 11 October 2009 over the South Atlantic Ocean and a high pressure system (1027–1033 hPa) situated to the north of the Iberian Peninsula that gradually moved further northward towards the British Isles. Most of the air masses observed on 12–13 October 2009, including all of those recorded during daytime, arrived at Badajoz from a Northeast-East (46° – 90°) direction. A small number of the air masses during 20:00–07:00 arrived from the East-Southeast (91° – 135°) (Fig. 5). Daytime and nighttime air masses spent similar amounts of time over the land and ocean during the episode. Air masses passed over the Spanish territory and so Portuguese source areas did not contribute to the overall load of airborne *Alternaria* spores recorded in Badajoz (Fig. 5). Two distinctive plumes of air masses were identified during the 48 h period (Fig. 6c–d). The source areas that most likely had an impact on airborne *Alternaria* spore concentrations recorded in Badajoz were crops and orchards located in Central Spain, e.g. the Castilla-León and Castilla-La

Mancha regions, as well as a minor contribution from grasslands situated in the vicinity of Madrid (Fig. 2).

3.3.3. Episode 3 (14–15 Jun 2010)

Adverse weather conditions in Spain dominated the third episode. The country was affected by two low air pressure systems, one originating from the South Atlantic and the other that moved northwards from North Africa to the Iberian Peninsula. Daytime and nighttime air masses arriving at Badajoz took similar paths. The most influential direction (Fig. 5) was found to be Northeast-East (46° – 90°). No contribution was recorded from a southerly arc ranging from East (91°) through to West (270°). The air masses spent slightly more time over the Atlantic Ocean compared to the land, with almost identical ratios recorded during the day and nighttime hours. The air masses (Fig. 6e–f) mainly passed over Spanish territory, with only a small contribution from source areas located in Portugal (8–9%) (Fig. 5). Potential sources of airborne *Alternaria* spores included crops, grasslands, and orchards in Northern Portugal and Northern Spain. The largest contributions were from crops and orchards with densities of 81–100% and 41–60%, respectively (Fig. 2).

3.3.4. Episode 4 (24–25 May 2011)

Good weather prevailed over Spain during 23–24 May 2011 due to the joint influence of two high air pressure systems (1023–1028 hPa). These high-pressure systems gradually weakened on 25 May 2011 in favour of a low air pressure system (997 hPa) that originated in the North Atlantic. During the episode, air masses approached Badajoz from Northeast–South directions (46° – 180°) during daytime, with East-Southeast (91° – 135°) being slightly dominant. Nighttime air masses were more variable, arriving from a wide arc from Northeast (46°) to Northwest (315°), although most (39%) air masses approached from a Northeast-East (46° – 90°) direction (Fig. 5). Daytime and nighttime air masses spent similar amounts of time over sea (52%) and land (48%). The vast majority of the time over the land was spent over Spanish territory (Fig. 5). Back trajectory analysis (Fig. 6g–h) shows that habitats in Central and Southern Spain were potential sources of airborne *Alternaria* spores, e.g. crops in Castilla-La Mancha and crops, orchards, and vineyards in Andalucía (Fig. 2).

4. Discussion

When identifying episodes of long-distance or regional scale transport it is important to have an understanding of the biology of the fungi being studied and the diurnal cycle of airborne fungal spore concentrations that can be attributed to local sources (Skj  th et al., 2012). Meredith (1963) examined the release of fungal spores from decaying banana leaves by representatives of the Deuteromycota group of fungi (Fungi Imperfecti), including *A. alternata*. The author showed that the regular diurnal periodicity in airborne spores displayed in dry weather (i.e. very low concentrations of spores between 18:00 and 06:00,

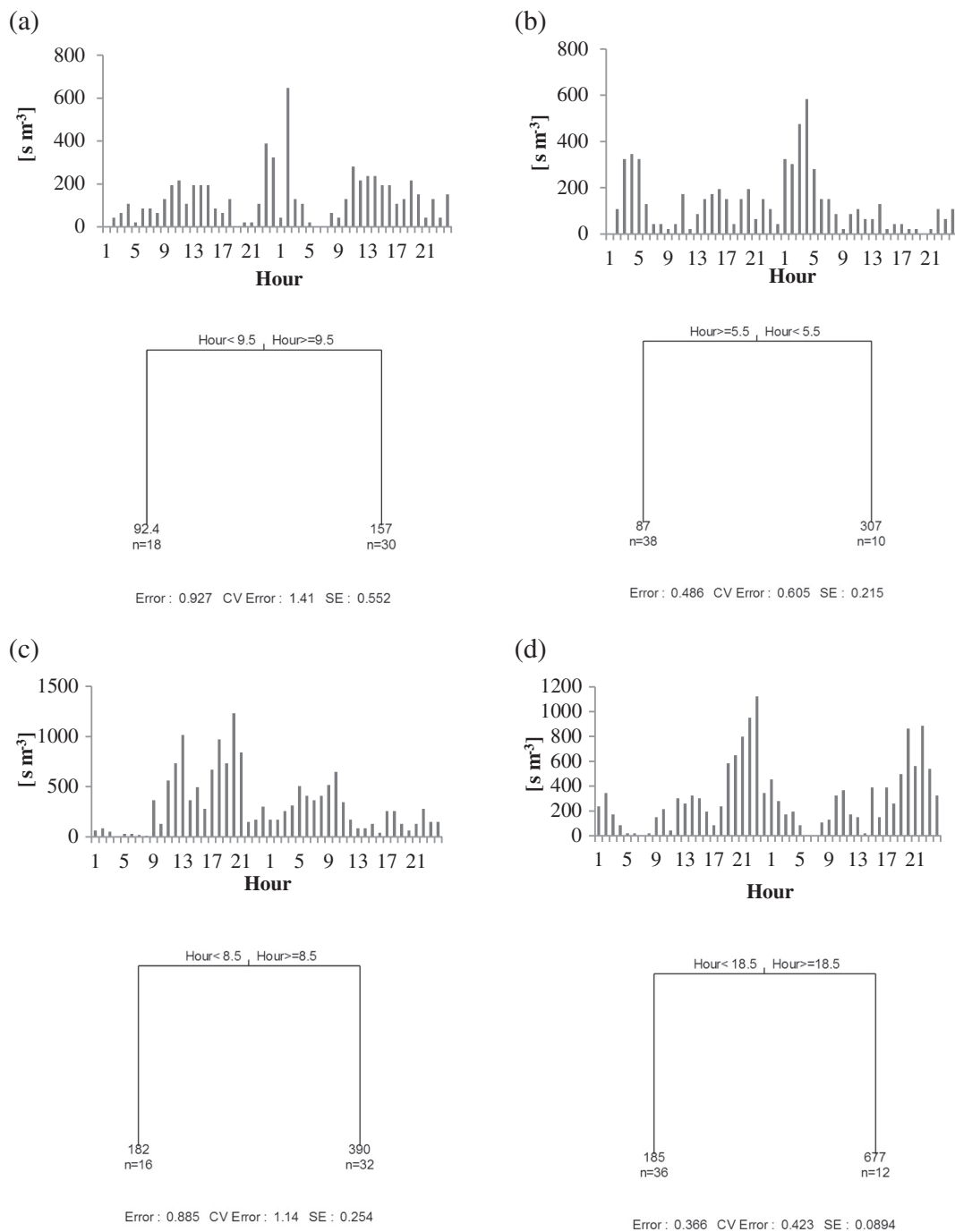


Fig. 4. Diurnal distribution of airborne *Alternaria* spores in Badajoz (above) and the results of multivariate regression tree analysis (below) for selected episodes: (a) 29–30 Jun 2009, (b) 12–13 Oct 2009, (c) 14–15 Jun 2010, (d) 24–25 May 2011.

followed by rapid spore liberation commencing around 07:00 that reached a maximum between 08:00 and 14:00) could be explained by environmental conditions; a rapid decrease in vapour pressure between 07:00 and 11:00 that triggered various spore release mechanisms (Meredith, 1962). Similar findings were reported by Rotem (1994), Angulo-Romero et al. (1999), Peternel et al. (2004), Rodriguez-Rajo et al. (2005) and Stepalska and Wołek (2009). Although no circadian pattern in airborne concentrations of *Alternaria* spores was reported by Sabariego et al. (2000).

Hourly concentrations of airborne *Alternaria* spores recorded in Badajoz during the examined episodes often peaked during the afternoon (09:00–12:00) and there were significant differences between atmospheric concentrations recorded during the day and night (Fig. 3).

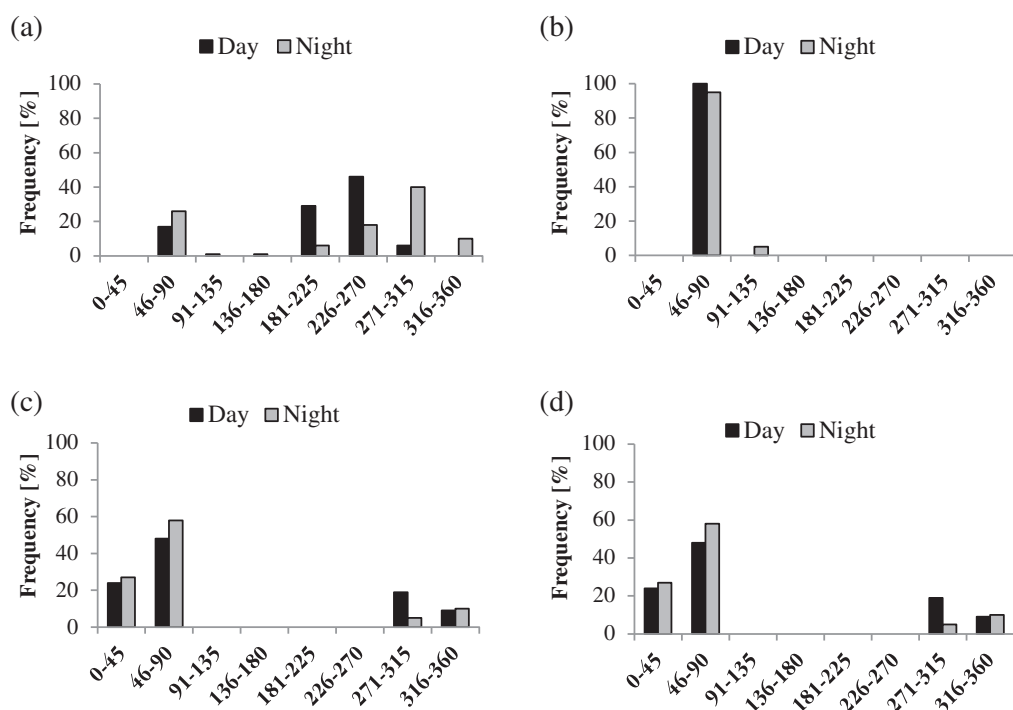
Savage et al. (2013) suggested that the liberation of fungal spores from mid- to late afternoon indicated a successful adaptation of a pathogen to the environment, because temperatures usually reach a maximum at this time and the warm air generates turbulent updrafts and the simultaneous increase of wind speed at ground level (Aylor, 1990; Savage et al., 2012). Nighttime peaks in airborne *Alternaria* spore concentrations, as seen on the 24–25 May 2011, cannot be explained by such environmental conditions and are therefore likely to be caused by different factors.

Meredith (1963) observed that decreases in relative humidity caused twisting of the conidial chains. Two or three seconds after this movement stopped, gas bubbles occurred that lead to breaks of the chain and conidia liberation (Meredith, 1963). Leach (1975) registered

Table 4The results of Spearman's rank test between hourly *Alternaria* spore counts and meteorological parameters recorded in Badajoz.

	Period	29–30 Jun 2009		12–13 Oct 2009		14–15 Jun 2010		24–25 May 2011	
		08–19 h	20–07 h	08–19 h	20–07 h	08–19 h	20 h–07 h	08–19 h	20–07 h
Mean air temperature	a	−0.329	0.315	0.537	−0.366	0.477	−0.056	0.508	0.904*
	b	0.050	0.293	0.623*	−0.352	0.663*	−0.063	0.361	0.914*
	c	−0.257	0.315	0.537	−0.394	0.589*	−0.063	0.424	0.494
Mean ground temperature	a	0.093	0.315	0.637*	−0.352	0.274	−0.063	0.301	0.914
	b	0.093	0.315	0.637*	−0.352	0.691*	−0.063	0.301	0.914
	c	0.054	0.315	0.637*	−0.352	0.477	−0.063	0.280	0.494
Relative humidity	a	0.289	−0.315	−0.456	0.408	−0.411	−0.268	−0.508	− 0.890*
	b	−0.050	−0.286	−0.548	0.521	− 0.621*	−0.014	−0.361	− 0.785*
	c	0.304	−0.315	− 0.584*	0.472	− 0.579*	0.042	−0.494	−0.494
Solar radiation	a	0.532	− 0.686*	0.185	0.022	0.407	0.296	0.438	0.618*
	b	0.629*	− 0.709*	0.185	0.117	0.460	0.283	0.431	0.640*
	c	0.557	−0.409	0.228	−0.196	0.775*	0.448	0.473	0.431
Wind speed	a	0.306	0.601*	0.576*	0.201	0.600*	0.028	0.364	−0.014
	b	0.000	0.119	0.481	− 0.665*	0.656*	0.471	0.529	0.658*
	c	0.139	0.442	0.551	−0.094	0.077	0.662*	0.522	0.270

a = concurrent meteorological data; b = 24 h lag; c = 48 h lag.

* statistical significance $p < 0.05$.

	Episode	Area							
		Ocean		Land		Country			
						Spain		Portugal	
		a	b	a	b	a	b	a	b
(a)	29–30 Jun 2009	67	73	33	27	7	3	26	24
(b)	12–13 Oct 2009	50	54	50	46	50	46	0	0
(c)	14–15 Jun 2010	53	54	47	46	39	37	8	9
(d)	24–25 May 2011	52	52	48	48	48	44	0	4

a = daytime trajectories (08:00–19:00)

b = nighttime trajectories (20:00–07:00)

Fig. 5. Frequency of air masses arriving at Badajoz from different directions within a spatial resolution $1^\circ \times 1^\circ$ and the time air masses spent above specific areas during the selected episodes (%): (a) 29–30 Jun 2009, (b) 12–13 Oct 2009, (c) 14–15 Jun 2010, (d) 24–25 May 2011.

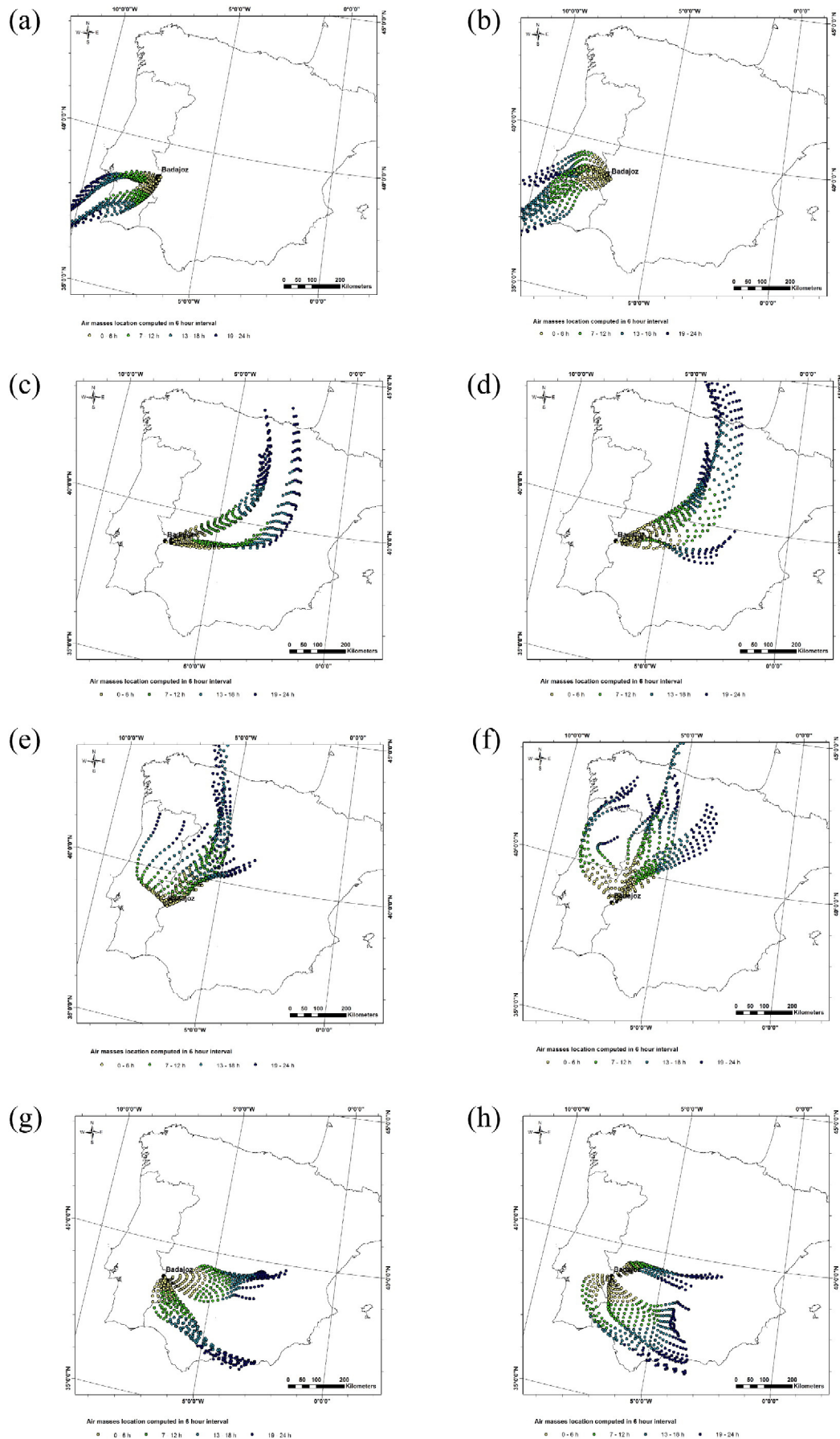


Fig. 6. Position of the day (left) and night (right) air masses during the following episodes: (a–b) 29–30 Jun 2009; (c–d) 12–13 Oct 2009; (e–f) 14–15 Jun 2010; (g–h) 24–25 May 2011.

changes in relative humidity either early in the morning or in the evening were also found to influence spore release by *Drechslera turcica* and several other fungal species that included *A. alternata*. In this article, the spore release in all of the examined fungi occurred when relative humidity was lowered, showing the “double maxima” pattern, with a greater peak in the morning and a lower peak in the evening. This was also reported by Maya-Manzano et al. (2012).

On the other hand, *Alternaria* conidia are firmly attached to the conidiophores and therefore spore liberation requires strong wind gusts (Rotem, 1994). Meredith (1966) found that the greatest concentration of *Alternaria porri* occurred when the wind speed was equal to 7 m s^{-1} regardless of the time of day or night, whilst Strandberg (1977) reported $2\text{--}3 \text{ m s}^{-1}$ for *Alternaria dauci*. Most of the fungal spores in general require at least 5 m s^{-1} to enable detachment of spores from conidiophores (Lin and Li, 2000). In the present study, results of Spearman's rank correlation analysis showed that wind speed was significantly related to airborne concentrations of *Alternaria* spores but not during every episode (Table 4).

Aylor (1999) estimated that diseases caused by fungi can be spread up to six times faster via long distance transport in a boundary layer compared to stepwise epidemiology observed locally at the field level. *Alternaria* spores have a dark wall pigmentation that protects against ultraviolet radiation (Rotem, 1994) whilst in the atmosphere. Ulevičius et al. (2004) found that sensitivity to solar radiation of various fungal spores, including those of *A. alternata*, varied depending on the time of day when spores were trapped; with the highest resistance to UV-B radiation exhibited by conidia that were collected during mid-afternoon hours (Ulevičius et al., 2004). The critical dose of solar radiation for *Alternaria* spores during cloudless skies in summer has been estimated as being 35 MJ m^{-2} (Rotem et al., 1985). With this in mind, Smith et al. (2012) reported that 47.6% of total captured fungal spores remained viable after being transported for 10 days by air currents in the troposphere (<6 km altitude) from Japan and China to North America. Out of three *Alternaria* species detected in the study, *Alternaria infectoria* was identified as being a foreign pathogen that requires monitoring (Smith et al., 2012).

For the period studied, the mean daily atmospheric *Alternaria* spore concentration was 31 s m^{-3} in Badajoz. During the same period, cities in the Central Spain, i.e. Albacete and Toledo (Castilla-La Mancha), recorded mean daily values of airborne *Alternaria* spores of 6 and 18 s m^{-3} , respectively (Sabariego et al., 2012). In the South of Spain, i.e. Málaga (Andalucía), the mean daily atmospheric *Alternaria* spore concentration was 13 s m^{-3} (Recio et al., 2012). It should be noted, however, that the peak days recorded in Albacete and Toledo for 2009 and 2010 were not the same as the peak days registered in Badajoz. Atmospheric concentrations of *Alternaria* spores in Badajoz will be influenced by distant and local sources, the relative contribution from these sources will depend on the strength of local sources and atmospheric conditions over the entire region that influence atmospheric transport.

The present study suggests that crops and orchards were the likely to be the main contributors towards airborne concentrations of *Alternaria* spores recorded in Badajoz. Potential source areas included crops in: southern Portugal and the Extremadura region in SW Spain (Episode 1); the Castilla-León and Castilla-La Mancha regions in Central Spain, (Episodes 2 and 4); and Northern Portugal and Northern Spain (Episode 3). Other potential sources were grasslands in Central and Northern Spain (Episodes 2 and 3) and Northern Portugal (Episode 3), and vineyards in Andalucía in Southern Spain (Episode 4).

Very few studies have examined the dispersion of *Alternaria* spores from crops such as cereals. One such study was conducted by Bashan et al. (1991) who reported that *Alternaria* spore concentrations decreased by 50% at a distance of 6 m from an infected plant in a cotton field, and no symptoms of infection were found at 20 m. Although Skjøth et al. (2012) projected that 5×10^{10} spores may be released per 1 ha during crop harvest, which could facilitate regional and long distance dispersal of spores such as *Alternaria*.

Concerning grasslands, Corden et al. (2003) found a statistically significant correlation ($r_s = 0.85$) between increased concentrations of *Alternaria* spores and wind originating from grassland areas at Cardiff, UK (1970–1996). The management of pastures and grasslands can have an influence on airborne fungal spores. For instance, Lacey (1975) found that *Alternaria* spores were more abundant in a hay area compared to a mown grassland area. Sreeramulu (1958) also found that, after mowing, concentrations of *Alternaria* spores increased by a factor of 4 above usual levels, rapidly reaching 1800 s m^{-3} , and took about 2 days to return to normal levels (Sreeramulu, 1958).

Plants belonging to the Poaceae family are grown for cereals and forage crops. In the study region, winter cereal (i.e. wheat sown in winter) is harvested from the end of June onward, whereas rainfed wheat and oats are harvested at the end of summer and in autumn. Corn and rice are also harvested in autumn. Some other cereals are used as forage (*Zea*, *Sorghum*, etc.) and fed straight to cattle. The vast majority of pastures in Extremadura are natural and grazed by cattle. Crops are rarely cut for silage in the south of Spain, although vetch (*Vicia sativa*) with oat (*Avena sativa*) and some white clover (*Trifolium* sp.) are sometimes grown and then cut at the end of spring (when the rains decline). It should be noted, however, that the harvesting of crops does not always result in elevated fungal spore concentrations. For instance, Maya-Manzano et al. (2013) found that levels of airborne *Alternaria* conidia were not directly affected by harvesting of the main irrigated crops – tomato and corn.

Studies conducted in vineyards by Diaz et al. (1998) and Magyar et al. (2009) showed that daily maximum concentrations of *Alternaria* conidia did not exceed 350 s m^{-3} . Furthermore, it can be postulated that a spore cloud, even one generated by a particularly virulent strain of *Alternaria*, would dilute its density with distance travelled from the inoculum source (Bashan et al., 1991). As a result, it is unlikely that vineyards could generate the levels of airborne *Alternaria* spores recorded in Badajoz, even if the vineyards were situated in close vicinity to the air sampler.

The identification of potential sources of airborne fungal spores can add valuable information about the aerobiology of a site. In this study, we present the novel use of habitat maps for orchards and vineyards. Previous studies only examined one or two vegetation types (Skjøth et al., 2012; Sadyś et al., 2014b), whilst this study provides a more thorough assessment of potential *Alternaria* sources.

5. Conclusions

The results of back trajectory analysis show that, during the examined episodes, the two main directions where *Alternaria* source areas were located were: (1) SW–W; (2) NW–NE. Land use calculated within a radius of 100 km from Badajoz shows that, during these examples of atmospheric transport, crops and grasslands are potentially the most important local sources of airborne *Alternaria* spores recorded at the site. Regional scale and long distance transport could therefore supplement the airborne catch recorded at Badajoz with *Alternaria* conidia originating from sources such as crops and orchards situated in other parts of Iberian Peninsula.

Acknowledgements

This work was enabled to conduct thanks to the Regional Government of the Junta de Extremadura in Spain (Project Number PRI06A190) and the European Social Fund (Project number PRI BS10008).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2015.06.031>.

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