The influence of meteorological parameters on Alternaria *and* Cladosporium *fungal spore concentrations in Beja (Southern Portugal): preliminary results*

Elsa Almeida, Elsa Caeiro, Ana Todo-Bom, Raquel Ferro, Ana Dionísio, Ana Duarte & Luiz Gazarini

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ORIGINAL PAPER

The influence of meteorological parameters on *Alternaria* and *Cladosporium* fungal spore concentrations in Beja (Southern Portugal): preliminary results

Elsa Almeida · Elsa Caeiro · Ana Todo-Bom · Raquel Ferro · Ana Dionísio · Ana Duarte · Luiz Gazarini

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Abstract *Introduction* Fungal spores constitute an important fraction of bioaerosols in the atmosphere. *Objectives* To analyse the content of *Alternaria* and *Cladosporium* spores in the atmosphere of Beja and the effect of meteorological conditions on their concentrations. *Methodology* The daily and hourly data of *Alternaria* and *Cladosporium* fungal spores concentration in the atmosphere of Beja were monitored from April 12, 2012 to July 30, 2014, based on the Portuguese Aerobiology Network methodology. The influence of meteorological conditions on the studied types of fungal spore concentrations was assessed through *Spearman's* correlation analysis. *Results* During the study period, 20,741 *Alternaria* spores and 320,862 *Cladosporium* spores were counted. In 2013, there were 5,822 *Alternaria* spores and 123,864 *Cladosporium* spores. The absolute maximum concentrations of *Alternaria* and *Cladosporium* spores were recorded on November 8, 2013, with 211 and 1301 spores/m^3, respectively. Temperature,

E. Almeida (✉)
Serviço de Patologia Clínica, Hospital José Joaquim Fernandes, Rua Dr. António Fernando Covas Lima, 7801-849 Beja, Portugal
e-mail: elsaalmeida.1983@gmail.com

E. Almeida · L. Gazarini
Universidade de Évora, Évora, Portugal
e-mail: gazarini@uevora.pt

E. Caeiro · R. Ferro
Sociedade Portuguesa de Alergologia e Imunologia Clínica – SPAIC, Lisbon, Portugal
e-mail: elcaeiro@yahoo.com

R. Ferro
e-mail: raquelsofferro@hotmail.com

A. Todo-Bom
Universidade de Coimbra, Coimbra, Portugal
e-mail: flcosta@netcabo.pt

E. Caeiro · R. Ferro · L. Gazarini
Instituto de Ciências Agrárias e Ambientais Mediterrânicas – ICAAM, Universidade de Évora, Évora, Portugal

A. Dionísio · A. Duarte
Hospital José Joaquim Fernandes, Unidade Local de Saúde do Baixo Alentejo, Beja, Portugal
e-mail: ana.dionisio@ulsba.min-saude.pt

A. Duarte
e-mail: crisduarte@sapo.pt

insolation and wind direction parameters showed a positive correlation with *Alternaria* and *Cladosporium* spore levels, while relative humidity and precipitation presented a negative correlation, which is statistically significant. Wind speed only showed a statistically significant positive correlation in terms of *Alternaria* spore levels. *Conclusion Alternaria* and *Cladosporium* spores are present in the atmospheric air of Beja throughout the year, with the highest concentration period occurring during spring and autumn. There was a clear effect of meteorological parameters on airborne concentrations of these fungal spores.

Keywords Fungal spores · *Alternaria* · *Cladosporium* · Meteorological parameters · Portugal

1 Introduction

According to Lacey (1981), atmospheric fungal spores originate from plants, animals and soil. The number of fungal spores in the atmosphere and their diversity vary throughout the day and based on season, geographical location and the presence of local spore sources (Aira et al. 2013).

Fungal spores represent a significant fraction of atmosphere bioparticles, which are usually detected at concentrations greater than 1000 spores day/m^3 (Oliveira et al. 2007; Nunes et al. 2008; Simon-Nobbe et al. 2008; Grinn-Gofron and Bosiacka 2015). Knowledge about the dynamics of the studied airborne fungal spores in a locality/region is of great interest not only in aerobiology, but also in the agricultural and health areas (Reyes et al. 2009). It is known that in the agricultural area some fungal species can colonise crop plants and damage them, leading to great agricultural and economic losses.

Studies carried out in the health area show that airborne fungal spores have important implications on respiratory allergic disease, where approximately 6% of the human population in developed countries exhibit sensitisation (Oliveira et al. 2005; Aira et al. 2013). In Portugal, according to a study by Aira and collaborators (2013), the sensitisation rate is about 3% and, in a European study, Heinzerling et al. (2009) described sensitisation values of 10.3%. Many studies consider *Cladosporium* as the most frequent and abundant fungal spore in atmospheric air around the world (Abu-Dieyeh et al. 2010). *Alternaria* is also associated with allergic respiratory diseases, such as rhinitis, allergic asthma and asthma exacerbation (O'Hollarem et al. 1991; Targonski et al. 1995; Kauffman et al. 1995; Damialis and Gioulekas 2006; Sabariego et al. 2007; Oliveira et al. 2007, 2009a; Simon-Nobbe et al. 2008; Heinzerling et al. 2009; Aira et al. 2012). In addition, the *Alternaria* fungus has been associated with significant damage in agriculture (Simon-Nobbe et al. 2008; Oliveira et al, 2009b).

Ianovici et al. (2013) cites Targonsky et al. (1995), who described the atmospheric concentration of 2000 spores/m^3 as linear to *Cladosporium* spores, cause clinical symptoms. With regard to *Alternaria* spores, Ianovici et al. (2013) cites Hasnain et al. (1998), who suggested that clinical symptoms occur with atmospheric concentrations higher than 50 spores/m^3.

As shown in the literature, the ecology of an area, climate factors, vegetation and microclimate(s), as well as human activities, may have a major influence on the seasonality of airborne spores (Abu-Dieyeh et al. 2010). There are not many studies in Portugal on the atmospheric aeromycological load, and no study regarding Beja is known. Therefore, it is of great interest to monitor the presence of fungal spores in the atmosphere of a city/region.

The objectives of this study were to identify and quantify the *Cladosporium* and *Alternaria* spores present in the atmosphere of Beja, as well as to analyse the influence of meteorological factors on their airborne concentrations.

2 Methods

The city of Beja is located in the Alentejo region, in the southern interior of mainland Portugal. The climate of the city is typically Mediterranean, with mild winters and long, hot summers (IPMA 2016).

In this study, the monitoring data of *Alternaria* and *Cladosporium* fungal spores present in the atmosphere of Beja, between April 12, 2012 and July 30, 2014, were used. For spore sampling, a Hirst-type volumetric trap (Burkard Seven-Day Recording Volumetric Spore Trap®) was installed in Beja's Hospital José Joaquim Fernandes (38° 00′ 54″N: 7° 51′ 47″W; 286 m above sea level), 30 m above ground level. The sample collection and analysis methodology was the

Table 1 Annual, maximum and average annual index of *Cladosporium* fungal spores counted during the several years of the study period (Apr 2012–Jul 2014)

	Total (spores)	Daily average (spores/m^3)	Maximum	
			Value (spores/m^3)	Date
2012	92,351	351	1273	24/05/2012
2013	123,864	340	1301	08/11/2013
2014	104,647	495	1966	07/04/2014

one used by the Portuguese Aerobiology Network (RPA-SPAIC) to monitor atmospheric allergenic pollen, based on the use of a silicone solution as an adhesive medium, the use of a solution of glycerogelatin with basic fuchsine as the colouring solution, the method of reading the 4 longitudinal lines by using an optical microscope with 400× magnification and the conversion of the counting results into the average number of fungal spores per cubic metre of air. This methodology meets the minimum requirements recommended by IAA (International Association for Aerobiology) and EAS (European Aerobiology Society) (Galán et al. 2014). The identification of fungal spores was based both on their morphological features (colour, size and ornamental features) and images from the literature (Smith 1984, 1986).

In order to determine the *Cladosporium* and *Alternaria* Main Atmospheric Spore Period (MASP), the 90% method was used. According to this methodology, MASP corresponds to the period in which the count of spore concentrations is higher than 5% and less than 95% (Grinn-Gofron and Bosiacka 2015). This denomination was considered to be the most accurate according to the study by Jato et al. (2006).

The intensity of the *Cladosporium* and *Alternaria* spore season was established by the number of days in which the average daily concentrations exceeded 2000 *Cladosporium* spores/m^3 and 50 *Alternaria* spores/m^3, threshold concentrations of airborne allergens cited by Ianovici and collaborators (2013), from which a high risk of exposure to these spores causing clinical symptomatology in allergic patients is considered to exist.

The hourly values of the meteorological parameters (temperature, insolation, relative humidity, precipitation, and direction and wind speed) regarding the studied period were provided by the Meteorology Department of Beja Air Base No. 11, which is located 8 km from the collector. Small variations in the meteorological conditions and atmospheric composition of the two sites are possible. However, the region has very similar landscape and climate features, and the use of hourly values counteracts this difference.

The effects of the meteorological parameters on airborne *Cladosporium* and *Alternaria* spore concentrations were assessed through Spearman's correlation analysis using Microsoft Office Excel 2007 and IBM SPSS Statistics 21.0.

3 Results

In general, the presence of *Cladosporium* spores in the atmosphere of Beja was observed throughout the analysed period, and a total of 320,862 spores were counted by using this methodology.

During the analysed period in 2012, 92,351 *Cladosporium* spores were counted, with the absolute maximum daily concentration at 1273 spores/m^3 on May 24. In 2013, 123,864 *Cladosporium* spores were counted, with a peak value of 1301 spores/m^3 on November 8. From January 1 to July 31, 104,647 *Cladosporium* spores were counted, with the maximum concentration at 1966 spores/m^3 on April 7.

The Main Atmospheric Spore Period (MASP) of the genus *Cladosporium* in 2013 began on February 1 and ended on December 11. In the year said, there was a daily average of 340 spores/m^3 in the atmosphere (Table 1).

In 2013, there was an increase in the concentrations of *Cladosporium* spores in June, July and October, and a decrease in February (Fig. 1). *Cladosporium* spores were never detected at a concentration greater than 2000 spores/m^3, and the lowest concentration cited in the literature is capable of triggering allergy symptoms (Ianovici et al. 2013).

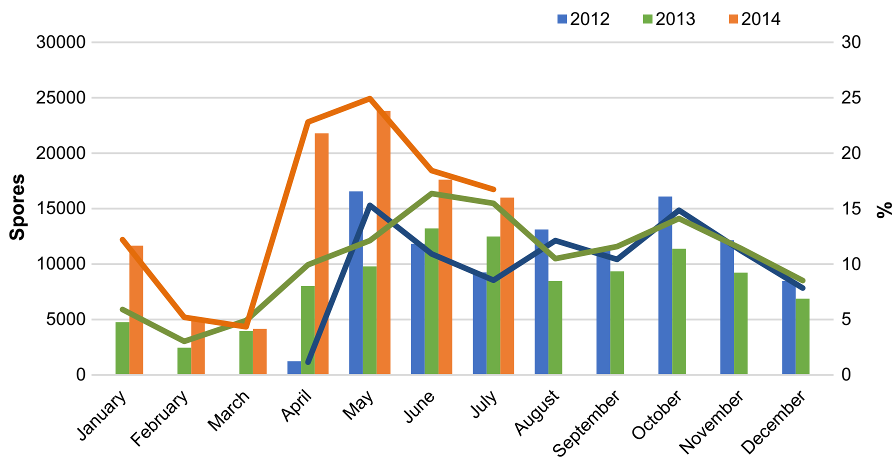

Fig. 1 Monthly absolute (represented in lines) and relative indices (represented in bars) of *Cladosporium* spores in the atmosphere of Beja during the study period

Table 2 Annual index, maximum daily and mean annual concentration of *Alternaria* fungal spores counted during the several years of the study period (Apr 2012–Jul 2014)

	Total (spores)	Daily average (spores/m^3)	Maximum	
			Value spores/m^3)	Date
2012	6306	24	158	15/06/2012
2013	5822	16	211	08/11/2013
2014	8613	41	333	25/06/2014

Regarding the *Alternaria* type, a total of 20,741 spores were counted in the analysed period.

In the period under analysis of 2012, 6306 *Alternaria* spores were counted with the absolute maximum daily concentration of 158 spores/m^3 on June 15. In 2013, 5822 spores of *Alternaria* were counted, where the average annual concentration was 16 spores/m^3 and the maximum observed was 211 spores/m^3 on November 8. In the analysed period of 2014, 8613 *Alternaria* spores were counted, with the peak value at 333 spores/m^3 on June 25 (Table 2).

The Main Atmospheric Spore Period (MASP) of the genus *Alternaria* in 2013 began on April 8 and ended on October 29.

Alternaria spores were low in the first months of the year. However, they increased during the spring, decreased in summer and increased once again in autumn. The highest monthly figure was recorded in October (Fig. 2).

There were 24 days in which concentrations of *Alternaria* were greater than 50 spores/m^3, whereby sensitised patients may exhibit clinical symptoms (Ianovici et al. 2013).

Table 3 presents *Spearman's* correlation coefficients between the meteorological parameters and the hourly concentrations of *Alternaria* and *Cladosporium* spores. Temperature, insolation and wind direction showed a statistically significant positive correlation with *Alternaria* and *Cladosporium* concentrations, while wind velocity showed a statistically positive correlation only for the *Alternaria* spore type. Relative humidity and precipitation showed a statistically significant negative correlation with both types of spores.

4 Discussion

This was the first aeromycological study carried out in the city of Beja. After comparing the atmospheric levels obtained from these two types, *Cladosporium* and *Alternaria, Cladosporium* levels were, as expected, much higher than those recorded for the

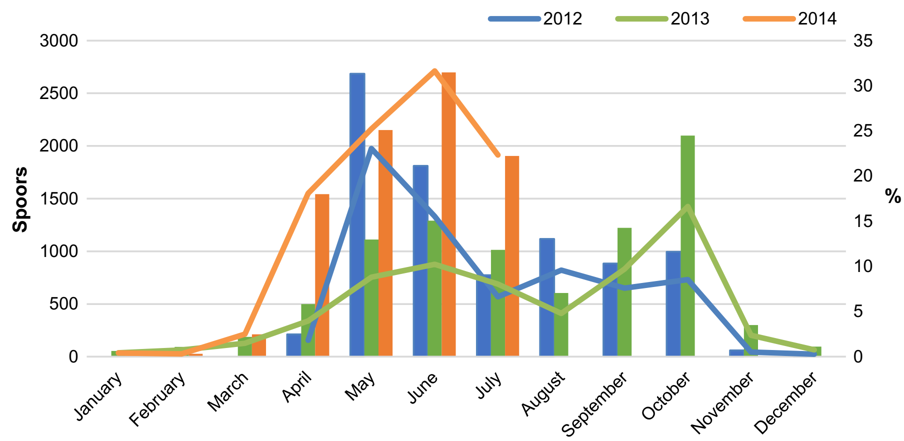

Fig. 2 Monthly absolute (represented in lines) and relative monthly indexes (represented in bars) of *Alternaria* spores in the atmosphere Beja during the period of study

Table 3 Spearman's correlation coefficients between the meteorological parameters and the atmospheric concentrations of *Alternaria* and *Cladosporium*

Fungal spores types	Temperature (°C)	Insolation (hha)	Relative humidity (%)	Precipitation (mm)	Wind speed (m/s)	Wind direction (°)
Alternaria	0.480***	0.131***	− 0.362***	− 0.122***	0.068***	0.161***
Cladosporium	0.321***	0.025***	− 0.210***	− 0.076***	0.012	0.050***

*0.05 ≥ p > 0.01; **0.01 ≥ p > 0.001; ***p ≤ 0.001

Alternaria type. Both types were present in the atmosphere throughout the year, especially in spring and autumn. It is characteristic of the Mediterranean region that *Alternaria* levels increase considerably in April to June and September to October (Maya-Manzano et al. 2012).

In 2013, *Cladosporium* spores were 123,864, with the maximum value being registered on November 8. *Alternaria* spores were 5822, and the maximum value was also recorded on November 8. The cities in the southern region of Portugal and inland cities generally present a higher concentration of fungal spores in the atmosphere (Nunes et al. 2008). The southern region's characteristic higher temperatures, its inland location and distance from the ocean may explain these results.

The *Alternaria* and *Cladosporium* spore concentrations presented a positive correlation that is statistically significant with temperature and insolation. Similar results were obtained in other Mediterranean cities such as Thessaloniki in Greece (Damialis and Gioulekas 2006), the Spanish cities of Valladolid (Reyes et al. 2009), Lugo, Santiago, Ourense, Vigo, Alcalá, Madrid (Oliveira et al. 2009a) and also Porto, in north Portugal (Oliveira et al. 2005). According to Linares et al. (2010), the optimal temperature for the development of *Alternaria* spores is between humid and mild climates. As for the *Cladosporium* type, the optimal temperatures are slightly lower than those of *Alternaria*, varying between 13 and 21 °C (Sabariego et al. 2000). When temperatures are lower or higher than previously described, the development, sporulation, release and dispersion of these spores are inhibited (Rodríguez-Rajo et al. 2005; Maya-Manzano et al. 2012). In Beja, temperatures during spring and autumn are mild and therefore, according to Oliveira et al. (2005), are favourable to the development of spores, thus justifying the high concentrations recorded during these periods of the year. On the other hand, when the average temperature is close to 10 °C in the winter and temperatures are above 30 °C in the summer, the spore levels present in the atmospheric air of Beja decrease. In addition to the development of the surrounding vegetation, among which cereal crops are found, the conditions are favourable for sporulation, release and dispersion of fungal spores (Oliveira et al. 2005; Abu-Dieyeh et al. 2010). The increase in atmospheric levels observed in the autumn (late September to October) may be due to the increase in dead matter, for example, caused by leaf fall and summer dry grass, an excellent substrate for fungal development and very important characteristic of this time of the year (Oliveira et al. 2005, 2007).

The atmospheric spore levels of *Alternaria* exhibit a statistically significant positive correlation with the wind parameter, speed and direction. With regard to *Cladosporium* spores, there was only a statistically significant positive correlation with the direction of the wind, which can mean that the production sources will be distributed in certain quadrant(s); on the other hand, the production sources are not close to the collector, but instead they are distant from it.

Precipitation showed a statistically significant negative correlation in *Alternaria* and *Cladosporium* levels. Results were similar to those obtained in studies conducted in Porto (Oliveira et al. 2007), Amares (Oliveira et al. 2009b), Santiago, Corunha, Viveiro, Lugo, Vigo, Ourense, Verín and Trives (Aira et al. 2008). Precipitation promotes the sedimentation of these fungal spore types and the subsequent decrease in their atmospheric levels (Aira et al. 2012).

A study carried out in the Portuguese cities of Porto and Amares on relative humidity showed a statistically significant negative correlation with *Alternaria* and *Cladosporium* levels as described by Oliveira and collaborators (2009a, b). Atmospheric *Alternaria* and *Cladosporium* spore concentrations increase when temperatures are mild and relative humidity is low. Herrero et al. (1996) described temperature values of 25 °C and relative humidity between 50 and 60% as the ideal meteorological conditions for the development of these fungi. Very low humidity values inhibit their growth. In this study, the atmospheric values of *Alternaria* and *Cladosporium* spores were highest in spring and autumn, with an average relative humidity of approximately 60% (Rodríguez-Rajo et al. 2005).

The difficulty in clearly understanding the established relationship between atmospheric levels of fungal spores and meteorological conditions may be related to small variations in the various meteorological parameters occurring throughout the day. Meteorological conditions influenced the atmospheric levels of fungal spores directly and/or indirectly, and it is not easy to analyse different meteorological factors separately, seeing that all of them simultaneously influence fungal development and the release, dispersion and transport of fungal spores in the air (Rodríguez-Rajo et al. 2005; Grinn-Gofron and Bosiacka 2015). According to Damialis and Gioulekas (2006) and other researchers (Kurkela 1997; Grinn-Gofrón and Strzelezak 2013), studies using daily averages will be subject to deviations caused by intermittent weather conditions. Therefore, in order to clarify the results obtained in this study, we use hourly values of the variables in the correlation analyses.

In spring, high levels of atmospheric pollen and *Alternaria* and *Cladosporium* spores occur simultaneously. The simultaneous presence of these particles in both atmospheric air and at high levels may lead to the worsening of allergic sensitisation and the subsequent aggravation of clinical symptoms (Damialis and Gioulekas 2006; Oliveira et al. 2007).

5 Conclusion

Cladosporium and *Alternaria* spores are present in the atmospheric air of Beja throughout the year. Atmospheric concentrations of *Cladosporium* and *Alternaria* spores vary throughout the time, being low during the winter and particularly high in two different periods of the year, namely spring and autumn. All meteorological parameters measured have a clear influence on the atmospheric concentrations of *Alternaria* and *Cladosporium* fungal spores. Temperature, insolation, speed and wind direction exert a positive influence, while relative humidity and precipitation exert a negative influence.

Since these types of fungal spores are an important source of aeroallergens responsible for allergic respiratory diseases in the Mediterranean region as well as important phytopathogens in other parts of the world, the monitoring of their atmospheric levels is considered to be critical, taking into account at all times that changes occur with variations in meteorological conditions, vegetation and crops (or agricultural methods).

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