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ORIGINAL PAPER

Aerobiology of Cupressaceae in Porto city, Portugal

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Abstract In the last years, pollen-related respiratory allergies have increased worldwide. In the case of Cupressaceae pollen, allergy symptoms appearing during the winter are often confused with generic respiratory diseases such as the common cold, influenza syndrome, among others. The aim of our study was to monitor the atmospheric of Cupressaceae pollen in the city of Porto, investigate its diurnal variation, as well as find the meteorological factors influencing its atmospheric concentration. Airborne pollen sampling was performed using a 7-day Hirst-type volumetric spore trap for 5 years (2013–2017). Cupressaceae main pollen season, interannual and diurnal variations, as well as correlations with the meteorological parameters were determined. During the study period, 2015 was the year that registered the highest annual pollen concentration, while 2016 presented the lowest. Cupressaceae pollen season occurred during the winter, being February the month with the highest concentration. The diurnal distribution pattern presents a well-defined peak concentration in the morning hours (9 a.m.–12 a.m.), which decays abruptly in the early afternoon. Finally, airborne pollen concentration and meteorological factors presented significant correlations, with temperature being the most influent variable.

Keywords Cupressaceae · Hourly distribution · Meteorological factors · Main pollen season

1 Introduction

During the last three decades, the incidence of pollen-related allergies had a dramatic increase worldwide, with 10–30% of the population being affected (D'Amato et al. 2010; Ring 2012). Allergic diseases caused by pollen include asthma, eczema, rhinoconjunctivitis, and urticaria, among others, affecting different organs, such as skin, eyes and the respiratory tract (Ring 2012).

In the Mediterranean countries, the prevalence of Cupressaceae species has increased mainly due to its use as ornamental plants, since they have low water needs, fast growth and have a low-cost maintenance (Charpin et al. 2017). These plants are anemophilous, shedding large amounts of pollen, being an important cause of allergic diseases, especially during the winter (Boutin-Forzano et al. 2005). However, the symptoms of allergies caused by Cupressaceae pollen can often mimic those of generic respiratory diseases, such as

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the common cold, influenza, among others, contributing to the undervaluation of Cupressaceae pollen as an important cause of allergy (Mari et al. 1997).

Aerobiological studies give important information to predict the length of seasonal exposure as well as the behaviour of pollen grains over the year (Martínez-Bracero et al. 2015), helping define the likelihood of human exposure to aeroallergens, sensitivity, and severity of allergic symptoms (Fuhrmann et al. 2016). In addition, they can be useful in climate change studies, allowing the monitoring and detection of changes in plant phenology, in determining the timing, intensity, and length of the main pollen season (MPS) as well as in the construction of pollen calendars. These model studies are important because they help patients and allergologists to prevent and properly treat allergies. Thus, this type of study should be taken into consideration by the local government in the construction of a garden or a green space in a city, since it avoids the implementation of plants that are potentially allergenic, improving the citizens' life.

The presence of pollen in the atmosphere is related to the flowering season of a given species and is highly influenced by meteorological conditions. The period in which pollen is present in the atmosphere is named as main pollen season, and its start and duration, as well as the number of pollen grains that may disperse in a specific region, are influenced by relative humidity, wind speed and direction, temperature, and rainfall duration (Gioulekas et al. 2004; Dahl et al. 2013; Hamda et al. 2017).

Several aerobiological studies have been performed in major Portuguese cities, such as Braga, Porto, Coimbra, Lisbon, Évora and Guarda (Ribeiro et al. 2003; Ribeiro and Abreu 2014; Lisboa et al. 2016; Camacho et al. 2017). The reported results showed temporal and quantitative prevalence differences in Cupressaceae airborne pollen concentrations. Despite that, Cupressaceae was one of the most representative pollen types in the atmosphere of these Portuguese cities, attesting the importance of monitoring its presence to improve the elaboration of pollen calendars and keeping them updated. This will enable the identification of Cupressaceae allergy symptoms in sensitized patients, facilitating proper treatment. In addition, these pollen calendars can also help patients avoid higher airborne pollen concentration periods, allowing them to take prophylactic measures (Šikoparija et al. 2018).

Ribeiro and Abreu (2014) performed the first long-term description of the allergenic airborne pollen present in the city of Porto and reported Cupressaceae pollen as the 5th most representative pollen type, pointing out to the importance of pollen allergy risk in this city. However, the widespread use of Cupressaceae plants as ornamental species in Porto has been increasing, in spite of a threefold increase in the percentage of cypress allergy around the Mediterranean area being reported (Charpin et al. 2017). The aim of this research was to perform a more detailed and updated study on Cupressaceae aerobiology in the city of Porto, in order to determine its MPS and to investigate its interannual variations and diurnal concentration of this pollen, as well as analyze the meteorological factors influencing its presence in the atmosphere.

2 Materials and methods

This aerobiological study was performed in the city of Porto, located in the Northwest of Portugal. Both Douro River and the Atlantic Ocean limit the city of Porto, the first at south and the second at west. During the study period, the average maximum temperature ranged between 11.5 and 21.0 °C and the average minimum temperature from 3.3 to 13.3 °C. Also, in this period the relative humidity varied from 47.1 to 96.5% and the annual total precipitation oscillated between 144 and 704 mm. In addition, during the winter, the winds are mostly from East and Southeast (Table 1).

The landscape around the airborne sampler is characterized by the presence of small gardens throughout the Faculty of Sciences of the University of Porto campus, the Botanical Garden, as well as avenue and street walks, which are ornamented with different trees. Around the sampling area, the most common Cupressaceae species found are *Chamaecyparis lawsoniana* (A. Murray) Parl., *Chamaecyparis obtusa* Siebold et Zucc. ex Endl., *Cupressus sempervirens* L., *Juniperus oxycedrus* L. and *Thuja plicata* "Zebrina" Donn ex D. Don. The sampling area is highly urbanized, and next to a highway that is one of the main entrances to the city.

Airborne pollen was continuously sampled from November 2012 to November 2017, using a 7-day Hirst-type volumetric spore trap (model Lanzoni

Table 1 Values of the meteorological parameters observed during the main pollen season of Cupressaceae in the atmosphere of Porto from 2013 to 2017

Year	*R* (mm)		$T_{average}$ (°C)			$T_{minimum}$ (°C)			$T_{maximum}$ (°C)			$RH_{average}$ (%)			$RH_{minimum}$ (%)			$RH_{maximum}$ (%)			WV (m/s)	
	Total	Max	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
2013	296.5	53.1	11.3	15.3	7.5	8.3	13.6	2.3	14.8	18.1	11.3	78.8	95.9	49.3	62.3	91.4	32.7	90.0	100.0	60.9	9.3	24.4
2014	704.1	115.0	12.0	18.0	7.9	9.0	13.2	3.8	15.3	25.3	10.5	81.2	96.1	37.3	63.8	89.2	20.5	92.3	98.0	68.1	11.0	19.6
2015	143.9	25.7	10.4	13.2	6.4	7.6	12.8	2.4	14.0	17.5	11.5	77.4	97.6	46.2	61.4	97.4	32.8	88.8	100.0	58.7	8.8	21.9
2016	431.2	61.9	11.4	14.6	6.9	8.3	13.4	3.3	15.2	19.7	11.2	79.5	97.3	52.3	62.0	93.2	30.0	90. 9	99.8	71.8	8.5	17.9
2017	295.7	60.7	12.8	16.7	8.6	9.8	13.7	4.6	16.4	24.3	12.9	79.4	95.7	50.5	62.4	89.5	31.9	90.9	97.4	67.5	9.4	19.3
2013–2017 Average	–	–	11.5	15.6	7.5	8.6	13.3	3.3	15.1	21.0	11.5	79.3	96.5	47.1	62.4	92.1	29.6	90.6	99.0	65.4	9.3	24.4

R rainfall (Max represents the absolute maximum value of daily precipitation registered during the main pollen season), *T* temperature, *RH* relative humidity, *WV* wind velocity, *Max* absolute maximum value registered among the daily records during the main pollen season, *Min* absolute minimum value registered among the daily records during the main pollen season

VPPS-2000, Italy). This trap is placed on the roof of the Faculty of Sciences of the University of Porto, approximately 18 m above ground level, and samples ten litres of air per minute, simulating human breathing. The air impacts onto a Melinex tape coated with silicone oil that is placed over a drum, which rotates once every 7 days. Every week the drum is replaced, and the tape is cut into seven daily segments and mounted on glass slides with fuchsine-stained glycerol jelly.

The daily and hourly number of Cupressaceae pollen grains per cubic metre of air were determined with an optical microscope (DMLB, Leica) at a magnification of 400 × along four full lengthwise traverses that are divided into 24 latitudinal sections of 2-mm intervals (each representing 1 h).

The Cupressaceae main pollen season (MPS) was defined using a pollen emission model, which fits a nonlinear logistic regression model to the accumulated sum of daily airborne pollen concentration sampled along a year. A one-sided *t* test was used at the 5% level to delimit the MPS beginning and ending dates, corresponding to the days where the difference between the pollen emission model and its superior and inferior asymptotes was significant (Ribeiro et al. 2007).

To determine the Cupressaceae diurnal airborne pollen concentration, the method described by Galán et al. (1991) was applied. The hourly values of atmospheric pollen concentration recorded in the days with an atmospheric pollen concentration superior to the average of the MPS concentration and without the occurrence of precipitation were used. The diurnal variations were expressed in percentage.

The presence of airborne pollen in the atmosphere highly depends on the meteorological conditions. Therefore, the meteorological data, namely maximum, minimum, and mean temperature and relative humidity, precipitation and wind speed, were correlated with Cupressaceae airborne pollen concentrations by applying the Spearman rank correlation test (significance level of 99%, 95% and 90%). The meteorological data were obtained from a weather station located at the Faculty of Sciences in Porto and managed by the Palynological laboratory.

Finally, the Cupressaceae daily pollen concentrations were classified into three different threshold classes according to the Portuguese Aerobiology Network (http://www.rpaerobiologia.com) that are indicative of the allergenic potential present in the atmosphere: low (1–30 pollen/m^3); moderate (30–60 pollen/m^3); high (> 60 pollen/m^3).

3 Results

The Cupressaceae main pollen season (MPS) in Porto city occurs from January to March (Table 2), being February the month with the highest recorded airborne pollen concentration (average of 214 pollen grain) (Fig. 1). Its duration varied over the years, between 1 month and almost 2 months, being on average 41 days.

During the study period, 2015 was the year with the highest annual airborne pollen concentration, attaining 952 pollen grains, while 2016 presented the lowest record, registering only 191 pollen grains (Fig. 2).

Daily Cupressaceae airborne pollen concentrations were above the high pollen threshold level defined (> 60 pollen/m^3) only on 10 days during the entire study period. Most of the days recorded a concentration below 30 pollen/m^3 (Table 2).

The diurnal distribution pattern of Cupressaceae pollen grains during the study period presents a well-defined peak concentration between 9 and 12 a.m., decreasing abruptly afterwards. The pollen concentration in the atmosphere remains low during the afternoon and starts to increase slowly during the night hours (Fig. 3).

Overall, the concentration of pollen in the atmosphere was positively correlated with the air temperature, being the maximum temperature the factor that most influenced the concentration of pollen in the atmosphere. Airborne pollen concentration was negatively correlated with rainfall during the 5 years of study, as well as with the relative humidity. The meteorological factors that least influenced the concentration of pollen in the atmosphere were wind velocity, as well as maximum and minimum relative humidity (Table 2).

4 Discussion

In Porto's atmosphere, the Cupressaceae main pollen season (MPS) occurs during the winter months, beginning, most of the years, in January and ending during March, in line to what has been reported in

Table 2 Main pollen season dates (Julian days from the 1st of January), duration, and pollen thresholds of Cupressaceae and Spearman correlation coefficients between airborne pollen concentration and meteorological parameters in the atmosphere of Porto from 2013 to 2017

Year	MPS (days)			Pollen thresholds (pollen/m^3 air)			Correlation coefficients (Spearman)							
				Number of days										
	Begin	End	Duration	1–30	30–60	> 60	R_{tot} (mm)	T_{max} (°C)	T_{min} (°C)	T_{mean} (°C)	RH_{max} (%)	RH_{min} (%)	RH_{mean} (%)	WV_{max} (m/s)
2013	22	68	46	147	3	1		0.398***					− 0.246*	
2014	33	71	38	116	7	1		0.428***	0.525***	0.506***				
2015	26	58	32	123	10	6		0.303*						
2016	25	81	56	128	0	0		0.275**						
2017	29	63	34	138	8	2	− 0.453***	0.604***		0.447***	− 0.367**		− 0.282*	− 0.379**
2013–2017	27	68	41	652	28	10	− 0.148**	0.278***	0.173**	0.201***		− 0.136**	− 0.130*	

Correlation is significant at *0.1, **0.05 and ***0.01 levels. R_{tot} total rainfall, *T* temperature, *RH* relative humidity, *WV* wind velocity, *max* maximum, *min* minimum

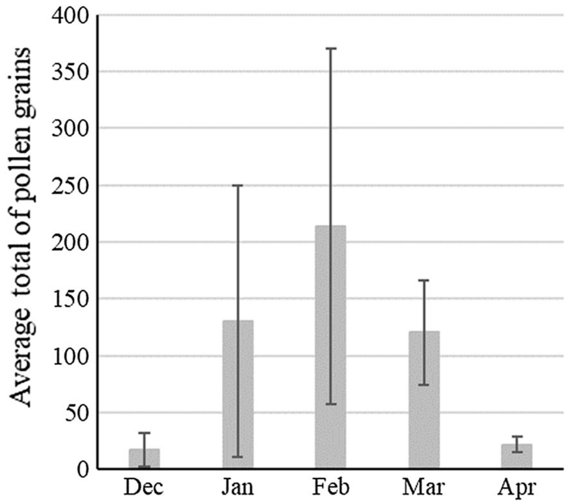

Fig. 1 Monthly average total pollen during the Cupressaceae main pollen season in the atmosphere of Porto from 2013 to 2017

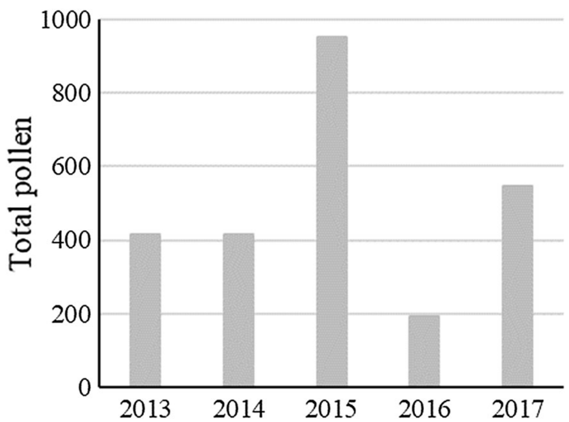

Fig. 2 Annual Cupressaceae airborne pollen in the atmosphere of Porto from 2013 to 2017

other European regions (Cristofori et al. 2010; Melgar et al. 2012). Nonetheless, there are some studies that recorded Cupressaceae pollen during autumn (Martínez-Bracero et al. 2015); however, the airborne pollen concentration found in this period of the year was smaller. Also, in a 1-year aerobiological study of the city of Guarda, in Portugal, the Cupressaceae main pollen season was reported to be between late March and early July, with a constant presence of this pollen type being observed in the atmosphere since December (Lisboa et al. 2016).

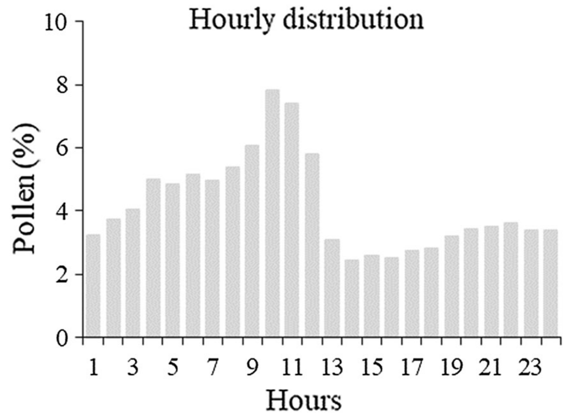

Fig. 3 Average hourly distribution, expressed in percentage, of Cupressaceae pollen concentration in the atmosphere of Porto from 2013 to 2017

In Porto, airborne Cupressaceae pollen appears in November and December but in very low concentrations, not contributing to the MPS. This happens because the Cupressaceae family includes several species that do not pollinate at the same time, presenting slight differences in the duration, beginning, and end dates of its flowering season (Boi and Llorens 2013). This fact contributes to the extension of Cupressaceae MPS when compared with other tree species in the same city (Ribeiro and Abreu 2014).

In 2015, the highest total annual airborne pollen concentration was registered with the shortest MPS, while 2016 registered the longest MPS along with the lowest total annual airborne pollen concentration. Usually, rainfall has a negative effect on the concentration of the pollen present in the atmosphere, since it washes out the particles present in it (de la Cruz et al. 2015). During the 2016 Cupressaceae MPS it rained in 64% of the days, but the highest rainfall levels were registered in 2014 (704 mm). Nonetheless, the total airborne pollen concentration was higher in comparison with 2016 (416 vs 191 pollen grains) possibly due to the fact that in 2014 the rainfall occurred mainly before and at the beginning of the MPS.

During the study period, the daily Cupressaceae airborne pollen concentration rarely exceeded the low threshold class of the allergenic potential present in the atmosphere (1–30 pollen/m^3). However, the rupture of the Cupressaceae pollen grain wall and the release of its cytoplasmic content were frequently observed, meaning that the allergens present in the submicroscopic sites of the exine and intine are readily available to the respiratory tract, increasing allergies to Cupressaceae pollen (Cresti and Linskens 2000). This phenomenon can occur after pollen hydration in the atmosphere. Also, in urban cities the interaction between Cupressaceae pollen grains and atmospheric pollution can increase the fragility of the exine causing several collapses and cracks, facilitating its break and shedding (Rezanejad 2009; Shahali et al. 2009). Besides, this interaction not only changes the pollen morphology but also its permeability, which facilitates the release and the fast diffusion of pollen allergens in the patients' nasal mucosa (Shahali et al. 2009). The allergenic potential of Cupressaceae pollen may be altered through the modifications that air pollutants may induce in the biochemical structure of the allergens or through changes in protein content (Gruijthuijsen et al. 2006; Sénéchal et al. 2015).

Concerning the pollen grains' diurnal concentration, it was reported in some studies that airborne pollen concentration is lower during the night than during the day (Ribeiro et al. 2008; de la Cruz et al. 2015). In our study, after the peak registered during the morning, Cupressaceae pollen concentration decreased in the afternoon, attaining the lowest values found throughout the day. Afterwards, airborne pollen concentration starts increasing in the late afternoon and continues during the night hours. Similar to our results, some authors registered elevated concentrations of airborne pollen at night (Ščevková et al. 2015; Grewling et al. 2016). This behaviour may be related to the convective heat transfer, which means that pollen grains are transported to higher tropospheric levels through convection currents during the day. During the night the air cools down and descends, transporting pollen grains to the ground level, leading to an increase in airborne pollen concentration (Grewling et al. 2016). Furthermore, as the evening starts, the atmospheric boundary layer compresses, which may lead to shorter convection currents, facilitating the accumulation of airborne pollen at ground level (Sofiev et al. 2013).

Similar to the findings of de la Cruz et al. (2015), in our study there were some years in which the concentration of airborne pollen was not correlated with rainfall, probably due to the constant rainfall that occurred during the MPS of each year.

Díaz de la Guardia et al. (2006) showed that temperature has a positive correlation with the concentration of airborne Cupressaceae pollen. Heat stimulates the opening of the anthers stimulating the release of pollen grains. In addition, when the climate gets warmer, the plants accumulate heat leading to the stimulation of flowering and, consequently, the increase in airborne pollen concentration (Aguilera et al. 2014), which might explain the positive correlations with the temperature obtained in this study.

5 Conclusions

Our study contributes with updated information about the Cupressaceae aerobiology in Porto city. Cupressaceae pollen is present in the atmosphere from late November to April, a consequence of the continuous flowering of several different species, and its levels rarely exceed the low threshold class of the allergenic potential present in the atmosphere (1–30 pollen/m^3).

Interannual variations in total airborne pollen concentration were observed. The pollen diurnal concentration patterns in the atmosphere present a well-defined peak concentration in the morning. Also, significant correlations between the airborne pollen concentration and the meteorological parameters were observed.

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