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Increased rupture of cypress pollen type due to atmospheric water in central and southeastern Spain

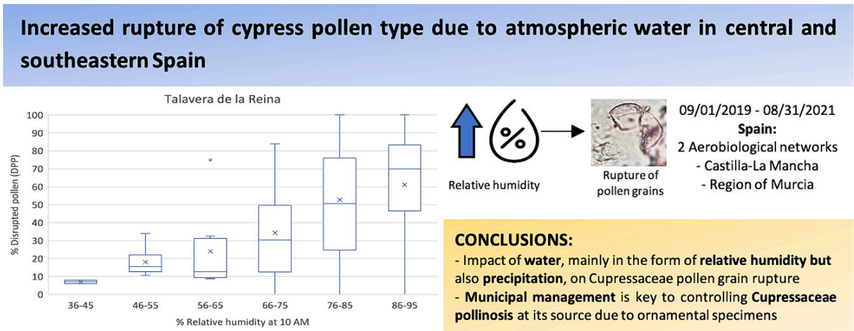
F. Aznar[a], L. Negral[a,*], S. Moreno-Grau[a], I. Costa[a], B. Lara[a,b], J. Romero-Morte[b,c], J. Rojo[c], R.M. Rodríguez-Arias[b], F. Fernández-González[b], R. Pérez-Badia[b], J.M. Moreno[a]

[a] Department of Chemical and Environmental Engineering, Technical University of Cartagena, Cartagena, Spain
[b] Institute of Environmental Sciences, University of Castilla-La Mancha, Toledo, Spain
[c] Department of Pharmacology, Pharmacognosy and Botany, Faculty of Pharmacy, Complutense University of Madrid, Madrid, Spain

HIGHLIGHTS

- Water content in the atmosphere explains Cupressaceae pollen disruption
- The higher the relative humidity, the greater the disrupted pollen concentration
- Blurred association between African dust outbreaks and pollen rupture
- Ornamental use of Cupressaceae overlaps natural specimens in pollen grain production
- Keys to prevent allergies: control urban flora and avoid high relative humidity days

GRAPHICAL ABSTRACT



Increased rupture of cypress pollen type due to atmospheric water in central and southeastern Spain

09/01/2019 - 08/31/2021
Spain:
2 Aerobiological networks
- Castilla-La Mancha
- Region of Murcia

CONCLUSIONS:
- Impact of **water**, mainly in the form of **relative humidity but** also **precipitation**, on Cupressaceae pollen grain rupture
- **Municipal management** is key to controlling **Cupressaceae pollinosis** at its source due to ornamental specimens

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ABSTRACT

This study aims to investigate the meteorological variables determining Cupressaceae pollen grain disruption in the environment. A parallel sampling of pollen grains and disrupted Cupressaceae pollen grains was performed in six cities using two Spanish aerobiological networks. The pollen concentrations, disrupted pollen concentrations, percentage of disrupted pollen and number of days when the percentage of disrupted pollen was above or equal to 50 % were quantified during two pollen seasons. The concentrations were determined following the standardised method EN 16868. Results show that the concentrations of pollen grains and disrupted pollen grains were not determined by geographical features and rarely by bioclimatic variables or indexes but by the ornamental use of the specimens in the vicinity of the pollen sampler, highlighting the possibility of using management practices to reduce exposure to allergens in the cities. African dust outbreaks coincided with higher concentrations of pollen grains and disrupted pollen grains, but the reduced percentage of disrupted pollen grains pointed to a non-

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Abbreviations: AEMET, Spanish Meteorological Agency; CLM, Region of Castilla-La Mancha; CPC, Cupressaceae pollen concentration; DP, disrupted pollen; DPC, disrupted pollen grains of Cupressaceae; DPP, disrupted pollen percentage of Cupressaceae; MPS, main pollen season; RM, Region of Murcia; SPIn, seasonal pollen integral.

[*] Corresponding author.

E-mail addresses: francisco.aznar@edu.upct.es (F. Aznar), luis.negral@upct.es (L. Negral), stella.moreno@upct.es (S. Moreno-Grau), isabel.costa@upct.es (I. Costa), Beatriz.Lara@uclm.es (B. Lara), jromer09@ucm.es (J. Romero-Morte), jesrojo@ucm.es (J. Rojo), rosamaria.rodriguez6@alu.uclm.es (R.M. Rodríguez-Arias), federico.fdez@uclm.es (F. Fernández-González), rosa.perez@uclm.es (R. Pérez-Badia), sele.moreno@upct.es (J.M. Moreno).

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causal relationship with long-distance transport. The effect of wind and maximum gusts remained negligible. The triggering factor for pollen disruption was the amount of water in the atmosphere, mainly reported as relative humidity. Rainfall increased the effect of disruption due to pollen grain swelling caused by its wash-out effect. The higher the relative humidity, the higher the disrupted pollen concentrations. This aligns with the mechanism of Cupressaceae reproduction since the family needs a water medium in the form of pollination droplets for the pollination tube to develop and the pollen grain to perform its biological function. Therefore, people that develop allergic symptoms to Cupresaceae pollen should avoid exposure during days with high relative humidity in the main pollen season.

1. Introduction

The Cupressaceae pollen type includes the pollen grains of species belonging to the Cupressaceae, Taxaceae and Taxodiaceae families since, from a morphological point of view, a higher taxonomic resolution is not reached (Green et al., 2004; Charpin et al., 2005; Bouchal and Denk, 2020).

Cupressaceae pollinosis is among the leading causes of pollen allergy worldwide (Charpin et al., 2019; D'Amato et al., 2007). Their prevalence is explained by the spatial and temporal distribution of their emission sources. If we look at the spatial distribution in Spain, this pollen type is released by seven autochthonous species of the *Juniperus* and *Tetraclinis* genera (Castroviejo et al., 1986), widely distributed in the Iberian Peninsula, but also by other allochthonous species. The intense and extensive ornamental use of cypresses (genre *Cupressus*), contribute to their increasing incidence in the pollen spectrum (Cariñanos and Casares-Porcel, 2011). In terms of temporal distribution, the Cupressaceae pollen type is the most frequent bioaerosol in winter in most cities with a Mediterranean climate (Velasco-Jiménez et al., 2020), and it has a very long pollination period due to the diversity of species comprising the pollen type (Charpin et al., 2019).

Cupressaceae pollen grains range from 15 to 35 μm in equatorial diameter. They are inaperturate, with usual Ubish bodies. These have narrow exine consisting of a granular ectexine and lamellar endexine, while the intine is coarser and fibrillar in appearance (Suárez-Cervera et al., 2003). The morphology of the Cupressaceae pollen grain resembles a disc at anthesis, and it is dehydrated (Chichiriccò and Pacini, 2008). During the reproductive process, hydration causes the pollen grain to pass through the micropylar canal into the micropyle when the pollen droplet is retracted (Takaso and Owens, 2008; Breygina et al., 2021). The presence of pores in Cupressaceae is disputed, with Charpin et al. (2019) stating that the pollen grain "is usually described as inaperturate, although a faint circular pore can be seen in fresh material". Kurmann (1994) were able to perceive what they called a "pseudopore" in a small percentage of pollen grains (Chichiriccò and Pacini, 2008).

Cupressaceae pollen grains are often observed with alterations in their structure, with pollen grain fragments, broken or open grains and other alterations (Shahali et al., 2009a), which we will call disrupted pollen (DP), found in aerobiological samples. This can be explained by the fragile configuration of the exine, which provokes more of the inner content of the pollen grain to be released after rupturing under stress (Shahali et al., 2009b; Sénéchal et al., 2015). The state of the pollen grain also depends on environmental conditions, with environmental humidity being one of the most relevant factors (Subba et al., 2023). Rezanejad (2009) observed that the intine swelled and the exine burst when the pollen grain was suspended in water, which is due to the hygroscopicity of the intine polysaccharides themselves. Rehydration experiments have shown them to be responsible for increased volume. The water content in the pollen grain of nearby coniferous pollen types is only 5–10 % (Owens et al., 1998). However, mature Cupressaceae pollen contains more water, around 30 %, making it a difficult pollen grain to preserve, especially when the relative humidity is above 10 % (Duhoux, 1982; Fernando et al., 2005; Ciani et al., 2020).

In the germination and appearance of the pollen tube of Cupressaceae pollen, the pore plays a fundamental role in regulating the water that enters the grain (Duhoux, 1982). This water causes the intine to swell and the exine to rupture (Rezanejad, 2009). The pore-controlled hydration mechanism occurs on the pollen droplet exuded by the ovule in successful reproduction. It has been argued that the composition of the pollen droplet may act selectively on pollen grains of the same species, ruling out cross-fertilisation (Gelbart and von Aderkas, 2002), and constituting an interesting ecological selection mechanism. Against this background, it is not surprising that the taxa whose pollen type we are dealing with are successful in water-stressed environments (Suárez-Cervera et al., 2008). Low relative humidity allows for easier transport of the dehydrated grain, while reproduction is only viable when it finds a specimen of the same species (Takaso and Owens, 2008).

A single pollen grain can release up to one million sub-pollen particles upon rupture (Wozniak et al., 2018). However, more paucimicronic particles in the air do not necessarily lead to increased allergen release (Cecchi et al., 2021). Buters et al. (2012) found that 90 % of the Bet v 1 allergen was in the aerosol fraction above 10 μm, highlighting the relevance of meteorological conditions for a higher concentration of allergens in the aerosol fraction below 10 μm, which is the size of sub-pollen particles. Therefore, a unique reproductive mechanism involving hydrating the pollen grain together with specific meteorological conditions could explain the large amount of Cupressaceae disrupted pollen grains in the ambient air and, by extension, the relevant pollinosis caused by this pollen type (D'Amato et al., 2007).

Studying pollen grain rupture in laboratory tests has attracted the attention of the scientific community in recent years (Visez et al., 2013; Cecchi et al., 2021). Galveias et al. (2021) conducted an observational study on the behaviour of Cupressaceae pollen grain rupture at an aerobiological sampling station in Évora, Portugal, during three-week events over two years. However, we are not aware of environmental studies at more than one site simultaneously, i.e. aerobiological networks, during the entire Main Pollen Season (MPS), which could reinforce the robustness of the results.

Simultaneously, exceptional meteorological events, such as intrusions of air masses from African deserts and long-distance transport, subject the pollen grain to stress and humidity changes, which are determinant factors for its rupture (Visez et al., 2015; Subba et al., 2023).

The aim of this paper is to study the influence of meteorological variables and dust outbreaks from African deserts on the presence and abundance of Cupressaceae DP grains compared to the total concentration of this pollen type present during long annual periods in the atmosphere of the central and southeastern Iberian Peninsula. The research aims to determine whether the bioclimatic conditions of the sampling stations and meteorological patterns, with special attention paid to dust outbreaks, influence the concentration of DP of a pollen type with a predisposition to breakage, such as Cupressaceae pollen.

2. Materials and methods

2.1. Study area, aerobiological networks and Cupressaceae main pollen season

Samples of airborne pollen grains were collected in six cities in the central and southeastern Iberian Peninsula, the sampling points of which belong to the two neighbouring aerobiological networks of the same area. We collected samples from the Aerobiological Network of Castilla-La Mancha (CLM) in the central Iberian Peninsula in the cities of Albacete, Talavera de la Reina and Toledo. In the Aerobiological Network of the Region of Murcia (RM), in the southeastern Iberian Peninsula, the sampling points were the cities of Cartagena, Lorca and Murcia. Geographical and bioclimatic information for the six sites is presented in Table 1 and Fig. S1 (Supplementary material).

The aerobiological sampling stations are in the western Mediterranean region, with one coastal station, i.e. Cartagena, and five inland stations with progressive continental indexes. Aerobiological samples were taken following the standardised method EN 16868 (2019). This method has been used for decades by the Spanish Aerobiology Network (Galán et al., 2007). The procedure is based on an active method of ambient air collection at 14.4 m^3/day, implemented in our case with a volumetric collector of the Hirst type, model Lanzoni VPPS 2000, which retains the airborne particles on a Melinex® tape impregnated with silicone placed on a rotating drum. This drum produces a continuous rotary displacement of 48 mm/day, which allows the tape fragments to be dated in the laboratory, where the sample is fixed and stained with glycerogelatin and fuchsin, respectively. This non-acetolytic procedure permits pollen types to be identified at 400 or 500 magnification. Counting pollen types on the tape requires a minimum surface of 10 % of the preparation to be read, in our case, on four longitudinal transects, which, when multiplied by a factor specific to each optical microscope, makes it possible to report the daily pollen concentrations in the ambient air as pollen grains/m^3. This method provides representative values of extramural pollen concentrations and it has been validated in interlaboratory trials in which our research groups have participated (Oteros et al., 2013, 2015).

The DP grains were counted following the pattern of rupture classes for *C. sempervirens* of Grilli Caiola et al. (2000). The daily pollen concentrations were used to determine the main pollen season (MPS) of the Cupressaceae pollen type calculated following Andersen (1991), which retains 95 % of the central concentrations of the distribution, excluding the tails of the days that add up to 2.5 % of the pollen grains at the beginning and the end of the flowering period. For each MPS, we worked with the concentration of (1) the total pollen grains of the pollen type, i. e. Cupressaceae pollen concentration, variable coded as "CPC"; (2) the disrupted pollen grains of Cupressaceae, "DPC"; and (3) the percentage of the latter compared to the sum of the whole and disrupted pollen grains, i.e. disrupted pollen percentage of Cupressaceae, "DPP". Following the recommended aerobiological nomenclature, we define the Seasonal Pollen Integral (SPIn) as the sum of daily concentrations throughout the MPS (Galán et al., 2017) (Table 2). For the calculation of the MPS, the beginning of the flowering period coincided with the beginning of the agronomic year, 1 September, and it ended on 31 August of the following year, in line with other works on Cupressaceae along the Iberian latitude (Belmonte et al., 1999; Aira et al., 2001; Tortajada and Mateu, 2008; De Linares et al., 2021). The study was

Table 1
Geographical and bioclimatic information on the pollen sampling stations and meteorological stations: a) Castilla-La Mancha and b) Region of Murcia.

a) Castilla-La Mancha

City	Albacete			Talavera de la Reina			Toledo		
Pollen sampling stations	38° 58′ 38″N 01° 51′ 27″W 686 m asl			39° 57′ 07″N 04° 50′ 38″W 371 m asl			39° 51′ 55″N 04° 02′ 30″W 559 m asl		
Meteorological stations	39° 00′ 20″N 01° 51′ 44″W 676 m asl			39° 57′ 31″N 04° 51′ 49″W 372 m asl			39° 53′ 05″N 04° 02′ 43″W 515 m asl		
Thermotypic horizons[a]	Upper Mesomediterranean			Middle Mesomediterranean					
Year[b]	2019–2020	2020–2021	1991-2021	2019–2020	2020–2021	1991–2021	2019–2020	2020–2021	1991–2021
Gorczynski index, K	34.2	40.9	35.3	37.6	37.8	29.9	37.9	43.2	35.9
Thermopluviometric continentality index, J	17.1	41.6	37.7	1.0	13.8	9.0	13.8	22.0	20.2
Thermicity index, It	303	240	271	359	323	279	323	250	288

b) Region of Murcia

City	Cartagena/San Javier Airport			Lorca			Murcia		
Pollen sampling stations	37° 36′ 18″N 00° 58′ 30″W 21 m asl			37° 38′ 42″N 01° 44′ 05″W 403 m asl			37° 58′ 57″N 01° 07′ 18″W 75 m asl		
Meteorological stations	37° 36′ 04″N 00° 59′ 16″W 17 m asl / 37° 46′ 42″N[c] 00° 48′ 21″W 4 m asl			37° 39′ 17″N 01° 41′ 14″W 312 m asl			38° 00′ 07″N 01° 10′ 15″W 61 m asl		
Thermotypic horizons[a]	Upper Thermomediterranean								
Year[b]	2019–2020	2020–2021	1991-2021	2019–2020	2020–2021	1991–2021	2019–2020	2020–2021	1991–2021
Gorczynski index, K	23.3	23.6	20.1	31.4	31.9	27.7	28.7	28.7	27.7
Thermopluviometric continentality index, J	2.8	3.9	3.2	2.5	11.5	26.7	4.2	20.1	16.4
Thermicity index, It	414	427	438	369	382	377	425	421	409

m asl = meters above sea level.

[a] Rivas-Martínez (1987).

[b] The indexes have been calculated as agronomic years (from 1 September to 31 August). 1998–2001, 2004–2009, 2011–2012 and 2015–2018 were not used to calculate the indexes in Talavera de la Reina due to a lack of records.

[c] All meteorological data for the city of Cartagena were provided by the Cartagena meteorological station, except the values of maximum and minimum pressure, which were taken from the San Javier Airport station.

Table 2

Characteristics of the Cupressaceae MPS in a) Albacete, b) Talavera de la Reina, c) Toledo, d) Cartagena, e) Lorca and f) Murcia.

Year	Onset date	End date	Peak date	Peak date count	MPS duration	SPIn Cupressaceae	Disrupted pollen			
	mm/dd/yy			Pollen grains/m^3	Days	Pollen grains/m^3	Disrupted pollen (DP) grains/m^3	% DP Mean ± SD	Days with DP ≥ 50 %	Ratio Mean ± SD
a) Albacete										
2019–2020	10/13/19	3/13/20	2/25/20	687	153	10,002	4582	46 ± 36	83	1.5 ± 2.4
2020–2021	11/11/20	3/31/21	1/30/21	820	141	5922	2895	49 ± 33	67	1.4 ± 2.8
b) Talavera de la Reina										
2019–2020	1/30/20	3/2/20	2/25/20	3394	33	21,421	8384	39 ± 26	17	3.4 ± 3.8
2020–2021	12/1/20	3/27/21	2/25/21	3748	117	37,373	10,036	27 ± 31	54	1.1 ± 1.2
c) Toledo										
2019–2020	1/10/20	5/3/20	2/29/20	865	115	7514	2423	32 ± 26	36	0.8 ± 1.1
2020–2021	11/22/20	3/27/21	2/20/21	1039	126	8412	2814	33 ± 27	41	0.7 ± 0.9
d) Cartagena										
2019–2020	9/24/19	4/27/20	2/26/20	386	217	5610	1681	30 ± 27	59	0.6 ± 0.9
2020–2021	9/25/20	4/17/21	2/13/21	116	203	3481	1205	35 ± 32	62	1.0 ± 2.0
e) Lorca										
2019–2020	10/10/19	4/24/20	2/21/20	1455	198	11,254	3006	27 ± 30	51	0.8 ± 2.3
2020–2021	11/1/20	4/17/21	4/1/21	533	168	7352	1704	23 ± 30	36	0.7 ± 2.4
f) Murcia										
2019–2020	10/12/19	4/22/20	2/23/20	556	194	9713	2713	28 ± 32	68	1.1 ± 3.2
2020–2021	10/24/20	4/18/21	4/1/21	223	177	4389	1062	24 ± 32	47	1.2 ± 4.3

mm/dd/yy = month/day/year; MPS duration = main pollen season duration in days; SPIn Cupressaceae = Cupressaceae pollen grains in the main pollen season; disrupted pollen (DP) grains/m^3 = disrupted pollen grains of Cupressaceae in the main pollen season; SD = standard deviation.

conducted simultaneously at the two regional aerobiological networks and comprised two MPSs for the years 2019–2020 and 2020–2021.

2.2. Meteorological and African dust outbreak information

The meteorological information was provided by the Spanish Meteorological Agency (AEMET). For each aerobiological sampling station, the variables of the meteorological station closest to its city were used (Table 1). Specifically, daily records were used for the following meteorological variables: precipitation (mm), mean temperature (°C), minimum temperature (°C), maximum temperature (°C), wind speed (m/s), maximum wind gust (m/s), maximum barometric pressure (mbar), minimum barometric pressure (mbar) and hourly relative humidity values (%). From these hourly relative humidity data, the daily minimum, maximum and average relative humidity levels were calculated.

Data on barometric pressure are not recorded by AEMET at the Cartagena, Lorca or Talavera de la Reina weather stations. For Cartagena, they were supplemented by the weather station at the nearby airport of San Javier (37° 47′ N, 0° 48′ W), a coastal town about 20 km from the city (Aznar et al., 2022; Negral et al., 2022). Bioclimatic indices and variables were calculated for each city in each flowering period: from 1 September 2019 to 31 August 2020 for the first MPS and from 1 September 2020 to 31 August 2021 for the second MPS. For the sake of comparison with a 30-year period and for reference purposes, the bioclimatic indices were also calculated for the 1991–2021 time series data.

Specifically, the bioclimatic indices were the Gorczynski index (Gorczynski, 1922), the Thermopluviometric continentality index (Valle Melendo, 1991), the Thermicity index (Rivas-Martínez et al., 2011, 2017) and the 19 BIOCLIM variables (Booth et al., 2014) available from WorldClim (Fick and Hijmans, 2017). All the details about the indices and their formulas can be seen in Table S1 and S2 (Supplementary material). Information on African dust outbreaks in the central and southeastern Iberian Peninsula was obtained from the report on these events prepared by the Spanish Ministry for Ecological Transition and Demographic Challenge (MITECO, 2022).

2.3. Statistical analysis

Daily aerobiological concentrations for each city were recorded in an Excel database. This was merged with the meteorological variables before being imported to the SPSS version 29.0 package, with which the statistical analysis was performed. First, the normal distribution of the aerobiological and meteorological variables was verified using the Kolmogorov-Smirnov test. Upon finding no normal distribution, logarithmic and square root (sqrt) transformations were performed. After ruling out normality with both methods, the Spearman coefficient was calculated between the aerobiological and meteorological variables for each city. After checking the lack of homogeneity of variance in the variables, the Kruskal-Wallis non-parametric test was applied to detect significant differences in the variables among the six cities. If significant differences were confirmed, Tamhane's post-hoc test was used to reveal between which city pairs these differences were detected. For the categorical variable African dust outbreak "Dust", the Mann-Whitney test was applied to test whether the selected variables adopted significantly different distributions on MPS days with and without African dust outbreaks. A hierarchical cluster analysis was applied following Ward's method to assess the grouping of the sampling points according to the following variables: indices and bioclimatic variables calculated for each flowering period, SPIn, peak day count (pollen concentrations on the peak day), MPS duration (days), DPC (sum of daily DPC data during MPS), DPP (mean daily DPP data during MPS) and number of days with disrupted pollen percentage $\geq$ 50 %. This method minimises intra-cluster variance and maximises homogeneity within clusters by pursuing the minimum sum of squared differences at the centroid (Trebuňa and Halčinová, 2012).

To visualise the effect of relative humidity on Cupressaceae pollen grain disruption, a box-and-whisker plot of the DPP as a function of relative humidity classification at 10 AM in the city of Talavera de la Reina was used for the MPS, as it is the city with the highest concentrations of the Cupressaceae pollen type. This time was selected because it showed the strongest correlation between relative humidity and DPP. These graphs have also been prepared for the other cities (Fig. S9). In addition, this type of graph has been used to represent the behaviour of the variables studied in the cities of the two networks.

3. Results

3.1. Cupressaceae main pollen season

Table 2 presents the dates of the onset, end and peak of the Cupressaceae MPS, the peak day concentration, the duration of the MPS and the SPIn. This table provides an overview of the key temporal and quantitative aspects of the Cupressaceae pollen season.

Fig. 1a shows the box-and-whisker plot of the CPC in the two aerobiological networks for the two years studied. The peak day in the cities of our networks accounts for 3.3–15.8 % of the SPIn (Figs. S2 to S7, in the Supplementary material).

3.2. Disrupted pollen data

Table 2 presents the sum of the DPC during the MPS, DPP and number of days the DPP exceeds or equals 50 %. The DPPs were higher in CLM [27 ± 31 %, 49 ± 33 %] than in the RM [23 ± 30 %, 35 ± 32 %]; the number presented in the intervals is the standard deviation. It should be noted that the lowest value in the CLM interval is form Talavera de la Reina, the city that recorded the highest absolute DPC value, 10,036 pollen grains disrupted/m^3, during the 2020–2021 MPS. The DPC and DPP showed significant differences when comparing the values of any city in one network with those of any city in the other network, except in the case of the DPC in the Lorca-Toledo pair, Lorca being the city with the highest DPC in the RM and Toledo the one with the lowest DPC in CLM. These two variables (DPC and DPP) outline the behavioural characteristics of the rupture in each network. Notably, the DPP most clearly shows the difference between the networks, as it is the only one of the three variables that presents statistically significant differences between and in the two networks.

Fig. 1b and c shows the box-and-whisker plot of the concentration of the DPC and DPP in the two aerobiological networks.

Table 3 shows the Spearman correlation coefficients between the CPC, DPC and DPP.

The Kruskal Wallis test with a p-value <0.01 indicates statistically significant differences between the means of the CPC, DPC and DPP in the MPS of the six cities. When this test is repeated for each region, only the DPP presents a statistically significant difference between the cities in the RM (p-value <0.05). In contrast, in CLM, all three variables present the following statistically significant differences: CPC and DPC (both with p-values <0.001) and DPP (p-value <0.05). Multiple comparisons with Tamhane's test show that Lorca and Murcia constitute a homogeneous group in all three variables for the cities in the RM. In CLM, Albacete and Toledo make up the homogenous group for the CPC and DPC, while for the DPP, the homogeneous group is Albacete and Talavera de la Reina. For the CPC, there are no significant differences between Lorca and the cities of Toledo and Albacete or between Murcia and Albacete, and for the DPC, no statistical significance is obtained in the difference between Lorca and Toledo. The DPP presents significant differences when comparing the values of any city in one network with those of any city in the other network.

Fig. 2 shows the dendrogram of the hierarchical cluster analysis for the SPIn of CPC for each year and city. The results of the hierarchical cluster analysis performed for all the variables defined in Materials and methods are shown in Fig. S8a–aa in the Supplementary material. The dendrogram illustrates how, initially, each element starts in its own cluster, and as they are grouped together, several distinctive subclusters are formed. The first one includes Toledo 1 and 2, Murcia 1, Albacete 1 and Lorca 2, and the second one groups Cartagena 1 and 2, Albacete 2 and Murcia 2. Talavera de la Reina, is a special case as it forms its own subcluster and remains separate until the last stage of combination, indicating a significant dissimilarity to the rest of the groups. The dendrogram allows us to observe the variability of the CPC between the different locations, showing that Talavera de la Reina presents a significantly different CPC from that of the rest of the cities.

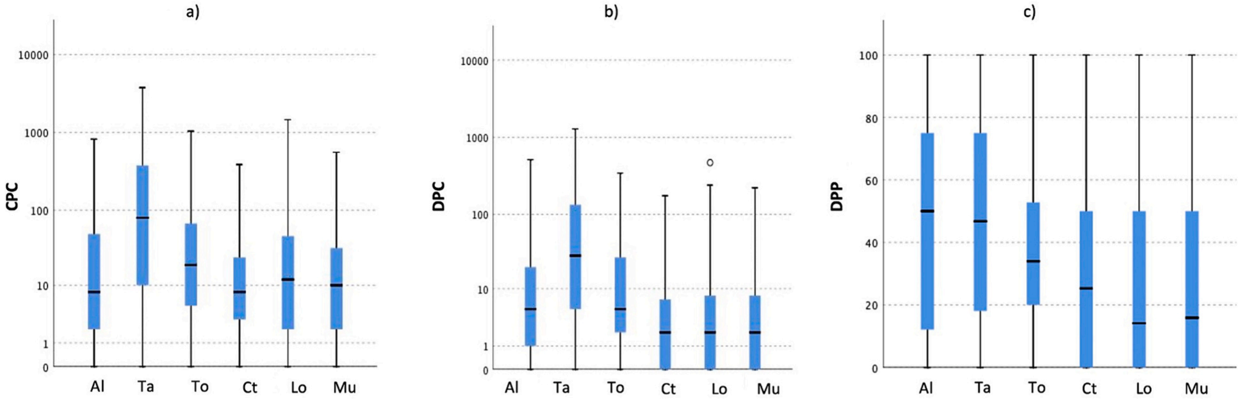

Fig. 1. Box-and-whisker plot of a) CPC, daily Cupressaceae pollen concentration, pollen grains/m^3 (log scale); b) DPC, daily disrupted pollen concentration of Cupressaceae, disrupted pollen grains/m^3 (log scale); and c) DPP, percentage of disrupted pollen grains of Cupressaceae, % in Castilla-La Mancha: Albacete (Al), Talavera de la Reina (Ta) and Toledo (To); and the Region of Murcia: Cartagena (Ct), Lorca (Lo) and Murcia (Mu).

Table 3
Significant Spearman correlation coefficients between Cupressaceae pollen concentrations and meteorological variables in Albacete, Talavera de la Reina, Toledo, Cartagena, Lorca and Murcia during the MPS.

	DPC	DPP	Prec	Tmean	Tmin	Tmax	Speed	Gustmax	Barmax	Barmin	RHmin	RHmax	RHave
Albacete													
CPC	0.955**	0.299**	−0.284**	0.517**	0.346**	0.512**	n.s.	n.s.	0.293**	0.273**	−0.341**	−0.233**	−0.362**
DPC		0.489**	−0.255**	0.516**	0.387**	0.479**	n.s.	n.s.	0.253**	0.238**	−0.264**	−0.175**	−0.273**
DPP	0.489**		n.s.	0.196**	0.216**	0.149*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Talavera de la Reina													
CPC	0.943**	−0.249**	−0.209*	0.405**	n.s.	0.616**	n.s.	n.s.	n.a.	n.a.	−0.357**	−0.172*	−0.336**
DPC		n.s.	n.s.	0.373**	0.167*	0.519**	n.s.	n.s.	n.a.	n.a.	−0.199*	n.s.	n.s.
DPP	n.s.		n.s.	n.s.	0.207*	−0.249**	n.s.	n.s.	n.a.	n.a.	0.403**	0.392**	0.460**
Toledo													
CPC	0.931**	n.s.	−0.226**	0.265**	n.s.	0.294**	0.138*	n.s.	0.276**	0.239**	−0.181**	n.s.	−0.180**
DPC		0.295**	−0.180**	0.209**	n.s.	0.209**	n.s.	n.s.	0.309**	0.269**	n.s.	n.s.	n.s.
DPP	0.295**		n.s.	n.s.	n.s.	−0.179**	−0.172**	−0.184**	0.162*	0.168*	0.257**	0.257**	0.286**
Cartagena													
CPC	0.779**	0.269**	−0.147**	0.338**	0.307**	0.318**	0.134**	n.s.	0.149**	0.113*	−0.124*	n.s.	−0.119*
DPC		0.713*	−0.120*	0.232**	0.211**	0.215**	n.s.	n.s.	0.183**	0.158**	n.s.	n.s.	n.s.
DPP	0.713*		n.s.	0.117*	0.117*	0.100*	n.s.	−0.112*	0.109*	0.107*	0.099*	0.124*	0.121*
Lorca													
CPC	0.760**	0.317**	−0.122*	0.433**	0.331**	0.425**	n.s.	n.s.	n.a.	n.a.	−0.123*	n.s.	n.s.
DPC		0.760**	n.s.	0.368**	0.346**	0.300**	n.s.	−0.140**	n.a.	n.a.	0.111*	0.155**	0.150**
DPP	0.760**		n.s.	0.235**	0.295**	0.129*	−0.160**	−0.229**	n.a.	n.a.	0.224**	0.178**	0.245**
Murcia													
CPC	0.782**	0.338**	−0.148**	0.523**	0.358**	0.517**	n.s.	n.s.	0.163**	0.152**	−0.156**	n.s.	−0.108*
DPC		0.753**	n.s.	0.440**	0.356**	0.407**	n.s.	n.s.	0.191**	0.190**	n.s.	0.108*	n.s.
DPP	0.753**		n.s.	0.281**	0.306**	0.205**	−0.106*	−0.171**	0.124*	0.142**	0.147**	0.178**	0.207**

CPC = Cupressaceae pollen concentrations. DPC = disrupted pollen grains of Cupressaceae. DPP = disrupted pollen percentage of Cupressaceae. Prec = precipitation. Tmean = mean temperature. Tmin = minimum temperature. Tmax = maximum temperature. Speed = average wind speed. Gustmax = maximum gust speed. Barmax = maximum barometric pressure. Barmin = minimum barometric pressure. RHmin = daily relative humidity minimum. RHmax = daily relative humidity maximum. RHave = average relative humidity. n.s. = non-significant correlation. n.a. = meteorological data not available.

[*] p-Value < 0.05.

[**] p-Value < 0.01.

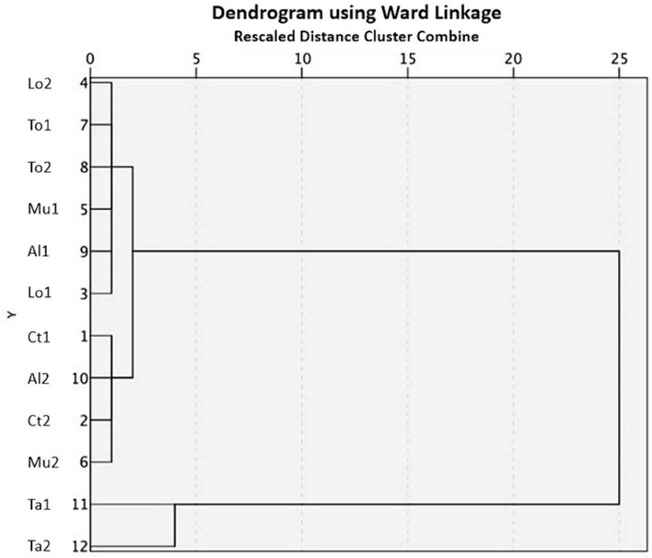

Fig. 2. Dendrogram of the SPIn of CPC with the records of Albacete (Al), Talavera de la Reina (Ta), Toledo (To), Cartagena (Ct), Lorca (Lo) and Murcia (Mu) in two main pollen seasons: 2019–2020 and 2020–2021. These main pollen seasons are coded as 1 and 2 after the city abbreviation on the Y-axis.

3.3. *Cupressaceae pollen, pollen disruption and meteorological events*

The concentrations of pollen grains, disrupted pollen grains and meteorological variables for each city of the two aerobiological networks are shown in Figs. S2 to S7 in the Supplementary material. The Spearman correlation coefficients between the CPC, DPC, DPP and meteorological variables in each city are also reported in Table 3. The dendrograms of the hierarchical cluster analysis for the above variables, based on the records of each of the two MPS, are represented for each city in Fig. S8 (Supplementary material). Fig. 3 shows the box-and-whisker plot of the DPP in Talavera de la Reina for the days of the study period grouped as a function of the relative humidity at 10 AM. Talavera de la Reina shows the clearest effect of relative humidity on DPP due to its high positive and significant correlation coefficient. The box-and-whisker plots for the other cities in the study are shown in Fig. S9.

3.4. *African dust outbreaks*

Table 4 shows the *p*-values of the Mann-Whitney *U* test between days with and without African dust outbreaks in the cities of the networks. The CPC, DPC and DPP increase significantly during the days with African dust outbreaks in all the cities except the DPC in Toledo, which does not reach statistical significance. The only cities where a decrease in the DPP is observed are Talavera de la Reina and Toledo.

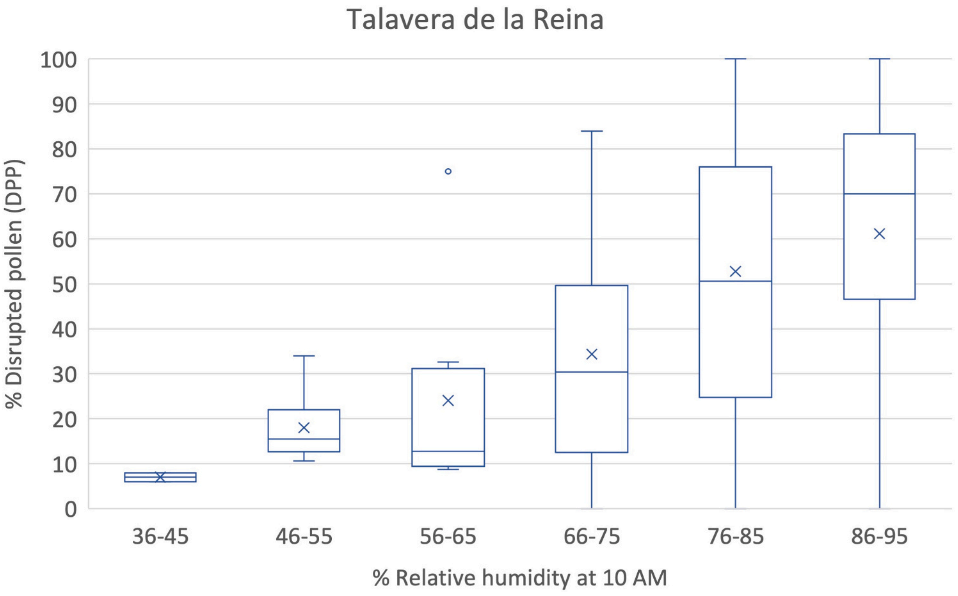

Fig. 3. Box-and-whisker plots of the disrupted pollen percentage (DPP) and intervals of relative humidity at 10 AM in Talavera de la Reina.

Table 4

Differences detected in the aerobiological variables between the days with and without African dust outbreaks in the cities studied: p-value of the Mann-Whitney U test.

	Albacete	Talavera de la Reina	Toledo	Cartagena	Lorca	Murcia
CPC	** ↑	** ↑	* ↑	** ↑	** ↑	** ↑
DPC	** ↑	** ↑	n.s.↑	** ↑	** ↑	** ↑
DPP	* ↑	** ↓	* ↓	** ↑	** ↑	** ↑

CPC = Cupressaceae pollen concentration. DPC = disrupted pollen concentration of Cupressaceae. DPP = disrupted pollen percentage of Cupressaceae. ↑ = increase in days with African dust outbreaks. ↓ = decrease in days with African dust outbreaks. n.s. = not significant.

[*] p-Value < 0.05.

[**] p-Value < 0.01.

4. Discussion

4.1. Main pollen season of Cupressaceae

In the years studied, the onset of the MPS in the six cities follows the pattern of higher thermicity, causing earlier flowering. In the CLM cities, the MPS begins between October and November. In the RM cities, the MPS begins between September and October.

Another aspect frequently indicated for the Cupressaceae pollen type is the prevalence of the specimens in the vicinity of the collector in the counts made (García-Mozo et al., 2016; Ciani et al., 2020, 2021), supported by the positive correlation between pollen concentrations and the flowering of nearby specimens of Cupressaceae (Monroy-Colín et al., 2020).

The sequence of the end date of the MPS reverses the spatial pattern described for its onset in the two regions. The MPS finishes earlier in CLM than in the RM. In the first case, it ends in March (except for one year in Toledo), and in the second case, it occurs in April. We find shorter MPS in CLM than in the RM. The relationship between the shorter duration of the MPS and its greater intensity has been reported elsewhere (Dahl et al., 2013). This relationship between the duration and intensity of the MPS was shown in Talavera de la Reina and Cartagena, the cities of each network with the longest MPS and SPIn.

The peak date usually occurs in February. An exception was 1 April 2021, coinciding in the RM in Lorca and Murcia. A similar behaviour was detected on the peak day, 20 February, in the CLM cities of Albacete and Talavera de la Reina in 2020.

The three cities of the RM are in a radius of 35 km in the thermotypic horizon Upper Thermomediterranean. The distance between Murcia and Cartagena is 45 km, less than the 60 km between the interior cities of Lorca and Murcia. With this shorter distance, it is worth wondering why there is no similarity with Cartagena within the network, which has its peak date a month and a half earlier. Factors that could influence this behaviour are the reduction in concentrations caused by the maritime influence (Negral et al., 2021) and the unequal urban use of a taxon introduced for ornamental purposes (Cariñanos et al., 2010).

As for Toledo's lack of similarity with the other two cities of CLM on the peak date of 2020, the gap is limited to a delay of four days in Toledo. The three stations are practically aligned on the same NW-SE axis of the southern sub-plateau of the Iberian Peninsula, with 275 km between Talavera de la Reina and Albacete. The latter cities share a thermotype but not a thermotypic horizon. This leads us to conclude that the peak date is due to local factors. Had it been caused by long-distance transport, distant inputs would have been detected almost simultaneously throughout the network (Negral et al., 2022) i.e. a large-scale event.

Therefore, we consider the impact of local *Cupressus* specimens for the two networks on peak date concentrations. The impact on the concentrations of Cupressaceae pollen caused by the ornamental and urban use of *Cupressus* specimens in the Mediterranean basin has been described in the scientific literature (Maya-Manzano et al., 2017; Cariñanos et al., 2019).

The local character of the concentrations of this pollen type is also reflected in the SPIn. In the SPIn of the same year, there may be 6-fold variations among the cities in CLM and 2-fold variations among cities in the RM (Table 1). The Kruskal Wallis test shows how the CPCs are statistically different in the six cities. In addition, the multiple comparison test indicates the homogeneous group of Albacete and Toledo in CLM, while in the RM, the cities of Lorca and Murcia constitute another homogeneous group, leaving Talavera de la Reina (CLM) and Cartagena (RM) outside these groupings. The disparity of pollen grain concentrations is even transferred to the hierarchical cluster analysis of the SPIn, in which the cities are grouped regardless of bioclimatic variables and indices (Fig. 2). Cartagena is isolated in a cluster with the second year of Albacete and Murcia. Consequently, within the two networks, our results confirm the importance of municipal policies regarding the selection and management of ornamental species to reduce the risks of pollinosis (Monroy-Colín et al., 2020).

4.2. Pollen disruption

The DPPs from the RM were lower than those reported by Galveias et al. (2021) in Évora [36 ± 19 %, 59 ± 23 %]; the statistic presented in the interval is the standard deviation, with only the DPP from Albacete remaining within this range. It is worth highlighting how these authors considered a typical day with a high percentage of disruption to be 42.5 % in 2017, while the following year, the typical day with a high percentage of disruption was 75.3 %. What was considered a typical day with low disruption in 2017 had a rate of 34.3 %, while in 2018, the typical day with low disruption had a rate of 23.2 %. Thus, the percentage of disruption during the MPS may not be fully indicative of increased and prolonged risks for the allergic population. Along this line, while there were only 6 and 19 days when the DPC was equal to or higher than 50 % in Évora, in our networks, there were between 17 and 83 days in CLM and between 36 and 68 days in the RM. Furthermore, the high values of the standard deviation for the DPP for each year point to considerable intra-annual variability. This suggests that some factors must be affecting pollen grain disruption on a daily scale, which is the time unit used to report pollen concentrations.

Talavera de la Reina shows a negative correlation between the CPC and DPP even though it recorded the highest amounts of pollen grains of the six cities. Although we cannot determine the cause, we have found some parallels between this fact and an in vitro hydration study of *C. arizonica* pollen grains (Chichiriccò and Pacini, 2008) in which, above a threshold concentration of pollen grains, the disruption was no longer detectable.

Variable environmental factors on a daily scale include meteors and atmospheric pollutants, which influence viability. Ramírez-Aliaga et al. (2022) reported that the viability of *Cupressus* pollen grains went from higher than 56 % in environments without stress factors, to lower than 40 % in environments with accentuated stress factors.

4.3. Cupressaceae pollen concentration (CPC), disrupted pollen concentration (DPC) and meteorological variables

Our study confirms the washout effect of rain on the CPC (Galán et al., 1998a; Aira et al., 2001, 2011; Green et al., 2004; Díaz de la Guardia et al., 2006; Ianovici, 2009; Makra et al., 2015; Rodríguez de la Cruz et al., 2015; Gomes et al., 2019). Although rainfall may prevent Cupressaceae pollination (Galán et al., 1998b), the negative correlation between the CPC and rainfall is not universal (D'Amato et al., 2018). The intensity and timing of rainfall in relation to the variables describing the MPS highly contribute to the statistically significant influence of rainfall (Ariano et al., 1994). In the case of leptokurtic distributions, the probability of rainfall having a significant impact is greatly reduced by rain on the peak date. This reasoning has been used to explain the lack of correlation between rainfall and Cupressaceae concentrations in the Iberian Mediterranean (Tortajada and Mateu, 2008), where, as in our case, fewer leptokurtic distributions are found in the MPS.

We must recognise that if most of our pollen concentrations come from ornamental specimens, managing these specimens is crucial to the allergenic potential beyond the washout effect of rain and other meteors. The relationship between the DPC and rainfall shows negative and significant correlation coefficients in Albacete, Toledo and Cartagena. On the one hand rain can wash pollen grains from the atmosphere regardless of their integrity. On the other hand, we know that rainwater, by hydrating and breaking the Cupressaceae pollen grain in the atmosphere (Gelbart and von Aderkas, 2002; Canini et al., 2004; Charpin et al., 2019; Gomes et al., 2019) increases the DPC (Galveias et al., 2021). In our opinion, the washout effect has been dominant over that of pollen disruption in Albacete, Toledo and Cartagena, the three cities where there is negative statistical significance between precipitation and the DPC.

The positive correlation of the CPC with temperature, especially the maximum temperature, is explained by the fact that maximum temperatures cause lower relative humidity, and this is when pollen is released from the anther into the atmosphere as dehiscence is triggered (Rodríguez-Arias et al., 2023).

Looking at the dendrograms of the bioclimatic variables in Fig. S8 (Supplementary material), it is precisely some of the variables related to temperature, i.e. BIO1, BIO4, BIO6 and BIO11, that create the first two clusters with the cities of each of the networks. Of these bioclimatic variables, we highlight the latter: the minimum temperature of the coldest month and the mean temperature of the coldest quarter, which correspond to the flowering periods of Cupressaceae described in our networks.

Regarding the relationship between temperature and DP, our results do not replicate those of Galveias et al. (2021), who reported a negative correlation between temperature and the disrupted pollen of Cupressaceae. In our networks, the pattern of correlations of the CPC with temperature is quite similar to that of the DPC with temperature, except for the minimum temperature in Toledo. As the temperature increases, we detect more broken pollen in the bioaerosol. We have seen that rain causes disruption, and high relative humidity promotes pollen grain disruption. Since high temperatures and the corresponding low relative humidity promote pollen grain release and discourage disruption, we could wonder when pollen grains are disrupted in our networks. In the winter context of our latitudes, this occurs from mid-afternoon onwards.

While in Talavera de la Reina and Toledo, the Spearman coefficient of the DPP with the maximum temperature is negative, this result shows a greater effect of temperature on pollen release than on pollen disruption. It would be reasonable to think that in these two cities, the temperatures would not have dropped sufficiently in the afternoon for the relative humidity to disrupt the pollen grains in the atmosphere. However, this has not been the case, as the lower temperatures are due to their continental character, as seen in the Gorczynski index (Table 1), and to the fact that the relative humidity of these two cities is among the highest at around 5–6 PM and at cooler times of the day (Table S3 in the Supplementary material). Considering the negative correlation between DP and temperatures, Hughes et al. (2020) have listed decreases in temperature and potential equivalent temperatures as a cause of increased paucimicronic particle release in the pollen grains of tree species.

Relative humidity is a determining parameter in the process of pollen disruption, as demonstrated by Tang et al. (2019) pollen grains suffer a significant increase in mass at high relative humidity. In Talavera de la Reina (Fig. 3), where the highest DPCs were found, at 10 AM, the higher relative humidity produces a higher DPP, reaching 70 % in the range of 86–95 % RH. Although this effect is easily observed in Talavera de la Reina due to the high concentrations of DPC, the Spearman correlation coefficients in the other cities are also positive and significant (Table S3). This indicates that relative humidity is equally determinant in pollen disruption in all the cities, although this effect cannot be so graphically observed in the other cities (Fig. S9). In those cities the best correlations were observed usually earlier than in Talavera de la Reina. The pollen's airborne journey is probably involved in the hour differences.

Due to the harmomegathy of the Cupressaceae pollen type, a process by which pollen grains change shape to adapt to volume variations caused by hydration changes (Punt et al., 2007), higher relative humidity leads to pollen grain weight gain due to the amount of water absorbed (Griffiths et al., 2012; Pope, 2010) and thus increases pollen grain deposition, which results in lower airborne concentrations. It has been documented that high relative humidity is necessary for the pollination droplet to remain above the ovule until it captures the airborne pollen grain, while low relative humidity causes droplet evaporation and attenuation of the pollen grain capture mechanism (Gelbart and von Aderkas, 2002).

Thus, low atmospheric humidity is necessary for dehiscence and pollen to be released from the anther (Rodríguez-Arias et al., 2023). With these premises, we can understand that in all the cities, the Spearman coefficients are nearly significantly negative. There is abundant literature referring to the negative correlation between relative humidity and pollen concentration in general and in particular that of the Cupressaceae pollen type (Aira et al., 2011; Sabariego et al., 2012; Rodríguez de la Cruz et al., 2015; Gomes et al., 2019).

To address the effect of relative humidity on the DPC and DPP, it is necessary to recall some milestones in the study the reproduction of the taxon, highlighting the pore-mediated mechanism of hydration and disruption. Duhoux (1982) carried out disruption tests with different aqueous solutions, proving that hypertonic solutions did not cause disruption. In CLM, the correlation of the DPC with relative humidity is negative because it would produce more pollen release from the pollen sac than the pollen grains it would break. In the RM, with fewer sources in these cities, the effect of relative humidity tends to promote disruption instead of inhibiting dehiscence. Clearly, this difference between networks disappears when looking at the correlation between the DPP and relative humidity. The weighting effect of the DPP shows that relative humidity promotes disruption in all the cities. Fig. 3 demonstrates that for the same time of day, the DPP rises with increasing relative humidity. Similarly, Hughes et al. (2020) recognise an increase in paucimicronic particles when relative humidity is high. Therefore, disrupted Cupressaceae pollen fragments under humid conditions represent a vector of allergens extrapolated to the upper respiratory tract (Canini et al., 2004). Having determined the influence of water, we can discuss its disrupting power, either as rain or relative humidity. We believe that while both promote disruption, the risk of exposure is likely to be higher in conditions of high relative humidity without rainfall (Rathnayake et al., 2017). In addition, many of the chemical reactions occurring in the atmosphere are favoured by water, causing pollutants to make the exine surface more fragile (Peltre, 1998; Charpin et al., 2019; Ščevková et al., 2020). High relative humidity could promote the chemical activity of aqueous-reinforced pollen grain aggressors, such as NO_2, which eventually fracture the grain or release large numbers of orbicules (Visez et al., 2013; Sénéchal et al., 2015; Charpin et al., 2019). Related to these arguments concerning the biotic aerosol, and together with other abiotic pollutants, high relative humidity could cause an increase in exacerbated asthma attacks and hospital admissions (Kassomenos et al., 2010; Makra et al., 2015).

Wind usually has little influence on Cupressaceae concentrations, as seen from the lack of correlation (Tortajada and Mateu, 2008; Gomes et al., 2019). Damialis et al. (2005) claimed that the lack of a relationship between wind and Cupressaceae pollen concentrations is due to the extensive local presence of Cupressaceae. Following this logic, we find that there is only a correlation with the wind modulus in the cities of each network with the lowest SPIn, i.e. a priori those with the lowest abundance of pollen type sources: Cartagena and Toledo. However, this is not confirmed by the maximum gust, which, conversely, is more energetic, even more dispersive, and may be strong enough to drag the pollen grains from these sources (which are not close) beyond the pollen trap. Furthermore, we have ruled out the relevance of turbulence resuspension due to wind gusts (Green et al., 2004). It should also be noted that in this study, we are only working with modules, not directions, which could lead to a clear relationship between geolocated specimens and the location of the collectors (Rojo et al., 2015). The fact that the DPC only correlates significantly with maximum gusts in Lorca attenuates the relevance of the two wind variables in our study in the disruption of Cupressaceae pollen (Galveias et al., 2021). With a negative Spearman correlation coefficient, there is no indication that wind gusts could resuspend DP to any noticeable extent (Rathnayake et al., 2017). It is true that if we look at the correlation between the DPP and the maximum gust, the significance increases to four cities, all of them negative. With these data, we have not observed evidence of mechanical disruption directly caused by wind. To address the negative correlation, we propose that the wind modulus, especially maximum gusts, dilutes the DP grains.

The uniformity of the positive and significant correlation of the CPC with barometric pressure is due to the atmospheric stability caused by high pressure. In our aerobiological networks, this results in higher temperatures, lower relative humidity and reduced mixing layer thicknesses, among other conditions (Salvador et al., 2019). Linking higher pressure and atmospheric stability with the previously discussed effects of other meteorological variables such as temperature, it is logical that the DPC, and even the DPP (except in Albacete), correlates significantly and positively with pressure. Furthermore, changes in barometric pressure influence not only the release of pollen but also the interaction of the pollen itself with the atmospheric pollutants acting on it, converting it into “polluen”, a term used to refer to atmospheric material that combines pollutants (gaseous and particulate) and allergens present in the air (Sénéchal et al., 2015).

4.4. Influence of African dust outbreaks

The presence of African dust outbreaks during the MPS coincided with significant increases in the CPC in all the cities of the two networks (Table 4). The impact of African dust outbreaks on pollen concentrations is an open topic of discussion. In León, in the northern Iberian subplateau, a decrease in Cupressaceae pollen-type concentrations has been observed on days with African dust outbreaks (Oduber et al., 2019). In cities in the southern Iberian Peninsula, García-Mozo et al. (2017) observed the relative importance of African dust outbreaks on *Olea* concentrations. In a previous study, pollen concentrations in general seemed to decrease during African dust outbreaks in the RM, while for CLM, concentrations behaved in the opposite way (Rojo et al., 2021). For CLM, incorporations of pollen grains could occur through the movement of African air masses over the Iberian Peninsula, while African dust outbreaks generally dilute the pollen load in the RM, as has been described for some abiotic aerosol components (Negral et al., 2008). This is in line with the results for CLM in our study but not for the RM, as Cupressaceae pollen concentrations increase. For *Olea* pollen, increased concentrations in the RM could be justified either by long-distance pollen grain transport events or by the promotion of pollen grain release, coinciding with African air masses (Negral et al., 2021). Unlike the olive tree, we have seen that Cupressaceae flowers in winter. In winter, African dust outbreaks are not as intense as in other seasons since they are mainly limited to a synoptic pattern of high African dust typical of winter in our area (Negral et al., 2012).

In Toledo, the presence of African dust outbreaks was not associated with an increase in the DPC of statistical significance. The rest of the cities had a statistical significance of 99 % for this association. The general explanation for this has been discussed as relating to the effect of temperature on the DPC. We have seen how the DPC in Toledo differed from that in other cities. The increased DPC could also be influenced by the chemical interaction of particulate matter and other pollutants with pollen (Behrendt et al., 1992; Rezanejad, 2009; Shahali et al., 2009a, 2009b; Caronni et al., 2021) or by the mechanical impact of particulate matter (D’Amato et al., 2007; Visez et al., 2015; Farah et al., 2020; Emmerson et al., 2021). We are cautious about this assertion, however, as we expected the wind to have some impact on promoting the breakup, and this did not occur in our networks.

During the African dust outbreaks, the DPP increased significantly to 99 % in the RM and 95 % in Albacete, while significant decreases occurred in Toledo and in Talavera de la Reina, with a statistical significance of 99 %. We have seen that in the case of the CPC, the influence is complex because aspects of local and regional transport come into play with long-distance transport. Although a significant influence of African dust outbreaks on the DPC is observed, more disruption of pollen grains subjected to greater transport in the atmosphere could be expected. The interaction with humidity and temperature produces contradictory results in different aerobiological stations in the central and southeastern Iberian Peninsula, as has been observed in the amount of total pollen (Rojo et al., 2021).

4.5. Limitations of the study

Zemmer et al. (2022) noted that MPS duration is determined by the method of definition. Using a method that retains 95 % of the central distribution, MPS durations of 89–137 days were obtained for Cupressaceae in Istanbul. This method resulted in durations of 97–100 days in Granada and 78–88 days in Cordoba (De Linares et al., 2021). Another method that retains the central 90 % of the distribution showed durations of 123–162 days in Valencia, 57–144 days in Toledo, 84–162 days in Salamanca, 29–143 days in Badajoz (Sabariego et al., 2012; Rodríguez de la Cruz et al., 2015; Silva-Palacios et al., 2016) and 32–56 days in Porto (Gomes et al., 2019). These methods may exclude important daily pollen concentrations in cities with high SPIn and platicuric distributions (Charpin et al., 2019). In our research, it results in the exclusion of days in CLM cities that, with the same concentrations, were not excluded in the RM cities.

Another limitation considered by the authors is the diversity of Cupressaceae species that contribute to the quantification of this type of pollen. In the study areas, different species may predominate, each with a different onset, end and duration of the MPS, which adds complexity to the analysis.

Last but not least, the limited temporal availability of data makes it more difficult to obtain general conclusions.

5. Conclusions

While the onset and offset dates of the MPS can be explained by latitude and altitude, the SPIn is independent of bioclimatic indices and variables due to the impact of ornamental specimens. Therefore, municipal management holds the key to controlling cypress pollinosis at its source, which confirms the importance of aerobiology in the context of sustainable cities following the urban agendas developed by the European Union.

The results have shown that rainfall and relative humidity are the most relevant variables in the disruption of the Cupressaceae pollen grain and that they are directly related to the plant's reproduction. While rain promotes disruption, it also has a washing-out effect on the atmosphere. And while high relative humidity inhibits dehiscence, it promotes disruption. It has been shown that higher levels of relative humidity are directly associated with a higher DPP, which is in line with the previous literature.

Since variations in relative humidity are linked to temperature, these two variables have been indispensable in understanding the dynamics of the environmental disruption of Cupressaceae pollen grains. The effect of relative humidity on pollen grain disruption is relevant for understanding the submicronic particles derived from the disruption of the exine in pollen grains, which has consequences for the allergic population, even when aerobiological networks report low concentrations of pollen grains in the air.

Finally, African dust outbreaks significantly influence the proportion of disrupted pollen grains in the air. However, although an increase in disruption during long-distance transport episodes is expected, this is not shown at all the aerobiological sampling stations due to the complexity of determining the local component during long-distance transport events. We propose that warm air masses from the African deserts have strongly motivated anthesis and not so strongly disruption. This is, therefore, an aspect to be investigated in future research based on the disruption of pollen grains in Cupressaceae or other taxa during other times of the year with a higher incidence of African dust outbreaks.

CRediT authorship contribution statement

F. Aznar: Supervision, Software, Methodology, Investigation, Conceptualization. **L. Negral:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Data curation, Conceptualization. **S. Moreno-Grau:** Validation, Supervision, Project administration, Investigation. **I. Costa:** Supervision, Investigation, Formal analysis. **B. Lara:** Writing – review & editing, Data curation. **J. Romero-Morte:** Writing – review & editing, Data curation. **J. Rojo:** Writing – review & editing, Supervision. **R.M. Rodríguez-Arias:** Writing – review & editing, Data curation. **F. Fernández-González:** Writing – review & editing, Supervision. **R. Pérez-Badia:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Data curation. **J.M. Moreno:** Writing – review & editing, Validation, Supervision, Project administration, Data curation, Conceptualization.

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

Data will be made available on request.

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Appendix A. Supplementary data

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