



Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Aerobiological importance and allergic sensitization to Amaranthaceae under arid climate conditions

Belén Elvira-Rendueles^a, Juan J. Zapata^b, Juan C. Miralles^c, José M. Moreno^a, Antonio García-Sánchez^a, Luis Negral^a, Stella Moreno-Grau^{a,*}

^a Departament of Chemical and Environmental Engineering, Technical University of Cartagena, Spain

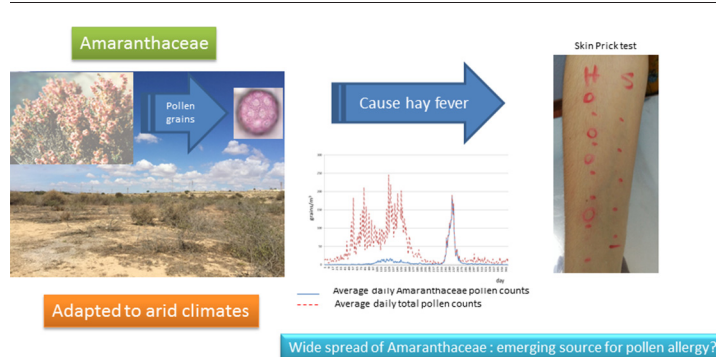
^b Private Allergy Clinic, Dr. Juan José Zapata Yébenes, Almería, Spain

^c Allergology Unit, Reina Sofía General Teaching Hospital, Murcia, Spain

HIGHLIGHTS

- Amaranthaceae is a specially adapted taxon to arid climates.
- Amaranthaceae pollen is the second cause of respiratory allergy in arid SE Spain.
- In summer/autumn the pollen counts of Amaranthaceae are important in arid SE Spain.
- New climate scenarios lead to the wide-spread of Amaranthaceae in arid zones.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 20 September 2016

Received in revised form 10 January 2017

Accepted 18 January 2017

Available online xxxx

Editor: D. Barcelo

Keywords:

Aerobiology
Amaranthaceae
Hay fever
Iberian Peninsula
Pollen counts
Skin prick test

ABSTRACT

Species of the Amaranthaceae family are abundant in the Southeast of Spain, one of the driest areas in Europe. The Amaranthaceae include species of interest from the point of view of allergic diseases. With the expansion of aridity, many species belonging to this family will be favoured. The objectives of this study were: first, to define the prevalence of sensitization to Amaranthaceae pollen in allergic patients in the Southeast of Spain; second, to present the aerobiological features of this taxon; and, finally, to demarcate those periods of increased risk of suffering allergic symptoms with the aim of improving the diagnosis and prevention of hay fever. Skin prick tests with extracts of pollen were performed on patients with symptoms of respiratory allergy referred for consultation for the first time in Almería and Murcia. Samples of the atmospheric bioaerosol were taken using a Lanzoni VPPS 2000 volumetric sampler, samples treatment and subsequent counts being carried out according to the methodology approved by the Spanish Aerobiological Network. The statistical significance of the correlation between aerobiological and meteorological data from this arid region in 2010–2014 was determined. The aerobiological study revealed the presence of two distinct peaks responsible for pollen symptoms. The flowering of this taxon is especially noteworthy in the city of Cartagena, where it supposes 94% of the total pollen collected in these periods. This situation converts Amaranthaceae pollen in the second leading cause of hay fever in the Southeast of Spain. With the expansion of aridity, an increase in the presence of these species is expected. Bearing in

* Corresponding author at: Departamento de Ingeniería Química y Ambiental, Universidad Politécnica de Cartagena, C/Dr. Fleming sn, 30202 Cartagena, Murcia, Spain.

E-mail addresses: belen.elvira@upct.es (B. Elvira-Rendueles), jzapata@upct.es (J.J. Zapata), jucarmir@telefonica.net (J.C. Miralles), sele.moreno@upct.es (J.M. Moreno), antonio.garcia@upct.es (A. García-Sánchez), luis.negral@upct.es (L. Negral), stella.moreno@upct.es (S. Moreno-Grau).

mind their prevalence in the spring and summer/autumn periods and their extensive presence in the bioaerosol, this makes data from Southeastern Spanish a benchmark with respect to the aerobiology of this type of pollen.

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1. Introduction

Hay fever or pollinosis is a disease with an environmental aetiology whose impact is related to well documented symptoms for the respiratory system. Although this disease was clinically and aetiologicaly described in the 19th century, there is abundant information on its occurrence since the Middle Ages (Pelta Fernández, 2005). The prevalence of seasonal allergic rhinitis is estimated to reach between 10% and 25% among the general population, with an increasing trend in recent decades (Navarro et al., 2009). The diagnosis of hay fever usually entails the following sequence: a detailed clinical history of the patient and the corresponding physical examination; the suspicion of a causal relationship between symptoms and exposure to allergens; and, finally, confirmation of the clinical suspicion by means of prick tests (Sánchez-Borges et al., 2011).

Inhaling pollen of the taxon *Amaranthaceae* is a major cause of respiratory allergy in the Midwest and Western United States, North Africa, the Mediterranean area (Villalba et al., 2014) and some Arabian countries (Al-Dowaisan et al., 2004; Assarehzadegan et al., 2009). The first case of a patient sensitized to *Amaranthaceae* was reported in Arizona in 1993 (Carnés et al., 2003). In Spain, these plants are widely found in the Southeast (Ferrer et al., 2010) and the Ebro Valley (Colás et al., 2005; Ferrer et al., 2012).

According to the Cronquist system (Cronquist, 1981), *Chenopodiaceae* and *Amaranthaceae* were considered two separate families. However, based on phylogenetic studies of molecular characteristics, taxonomists nowadays classify what was termed *Chenopodiaceae* as a subfamily of *Amaranthaceae*. Hence, the correct name of the family from the botanical point of view is *Amaranthaceae* (Bremer et al., 2009; Pérez-Badía, 2015). Nonetheless, some botanists believe that the merging of these two families is unclear (Kadereit and Freitag, 2011; Pérez-Badía, 2015).

The family *Amaranthaceae* includes >2000 species (Stevens, 2001). Overall, it comprises annual or perennial grasses that are well represented in the Southeast of Spain due to the optimum conditions they encounter in arid and semiarid climate zones. They are also found in special habitats such as halophyte areas, salt marshes, gypsum formations, and the like.

In the Region of Murcia, 19 genera (58 species) can be found to occur naturally, while 17 genera (43 species) can be found in Almería (Anthos, 2016). Among these, the following may be highlighted (Castroviejo, 1986–2012) for their botanical (Pérez-Badía, 2015) and clinical (Huertas, 2013) importance:

Genus *Salsola*: *S. oppositifolia* Desf., *S. genistoides* Juss. ex Poir., *S. kali* L., and *S. soda* L., which abound in scrubland on loamy, alkaline or subsaline soils, halophyte plants called *almarjo* or *barrilla* that at one time served as raw material for the production of carbonates and soda.

Genus *Chenopodium*: *Ch. murale* L. and *Ch. album* L., typically found in ruralized areas, roadsides, abandoned fields and walls.

Genus *Atriplex*: *A. halimus* L. and *A. glauca* L., found in sandy and clay soils with salinity.

Genus *Salicornia*: *S. ramosissima* Woods, typically found in coastal waterlogged salt marshes and salt flats and inland brackish or saline lagoons.

Genus *Halimione*: *H. portulacoides* (L.) Aellen, found in marshes and flooded coastal land.

Genus *Amaranthus*: *A. muricatus* (Moq.) Hieron. and *A. viridis* L., which are found in nitrophilous, roadside, ruderal and cropland communities.

There are also genera that are grown for their agronomic (*Beta*, *Spinacia*, etc.) or ornamental (*Amaranthus*, *Celosia*, etc.) value (Cariñanos et al., 2014).

All species of *Amaranthaceae* share the same pollen type. They are pantoporate, with >20 pores, which are non-polar, radially symmetrical, spherical and small to medium in size (20–29 µm in diameter) (Galán Soldevilla et al., 2008; Pérez-Badía, 2015). The number of pores distributed over the surface can vary from 30 to 60, presenting a diameter of 2 to 3 µm. The apertural membrane is covered with numerous granules and spikes. The exine is 2 to 3 µm thick, with a rugulate-scarbid or echinulate surface. They present a complete tectum and columellar infratectum (Candau, 1987).

The biogeographical delimitation of the area of Almería and Murcia (Alcaraz et al., 1991; Rivas-Martínez, 1987) to the subsector level corresponds to the following hierarchy: Boreal Kingdom; Mediterranean Region; Western Mediterranean Subregion; Mediterranean-Ibero-Levantine Superprovince; Murcian-Almerian Province.

Although pollen grains can be transported over long distances (Moreno-Grau et al., 2016), the amount collected at any aerobiological monitoring point depends largely on the nearby plant biomass, reflecting the qualitative and quantitative content of the characteristics of bioclimatic areas modified by anthropogenic factors. Moreover, the production of pollen grains is regulated by both intrinsic and extrinsic factors (Cariñanos et al., 2014). Among these, environmental factors are to be highlighted, with temperatures and rainfall being of special importance (Cariñanos et al., 2004; Fernández-Montes and Rodrigo, 2015).

As the area under study corresponds to one of the driest in Europe, many genera of this family find it to be an ideal habitat for their development. Their presence in the area facilitated the development of a major industry and hence many species were cultivated in the past. It should not be forgotten that many of the plants known locally as *barrillas* or *almarjos* (*Halimione portulacoides* (L.) Aellen, sea purslane; *Sarcocornia fruticosa* (L.) A.J. Scott, saltwort, samphire; *Halogeton sativus* (Loefl. ex L.) Moq., Alicante barrilla; and *Salsola soda* L., opposite-leaved saltwort) constituted the raw material for obtaining carbonate before the 19th century, besides being an important source of wealth (Moreno-Grau, 2006).

From a clinical point of view, the most relevant genera of the family *Cupressaceae* in the Mediterranean area are *Salsola* and *Chenopodium* (Villalba et al., 2014). The aetiological diagnosis of hay fever is performed via prick tests employing aeroallergen extracts. At present, the pharmaceutical industry provides standardised extracts of *Salsola* and *Chenopodium*. To the best of our knowledge, nine allergens have been identified in *Amaranthaceae*, three of which are due to *Chenopodium album* L., five to *Salsola kali* L., and one to *Amaranthus retroflexus* L. (Villalba et al., 2014). As the *Amaranthaceae* pollen type is usually found in low concentrations when numerous pollen counts are carried out, the role of these allergens as the cause of hay fever has been questioned (Moral de Gregorio et al., 2016). Nevertheless, their low concentration in air is sufficient to produce high skin sensitization (Pola Pola et al., 1998). This pollen type is becoming increasingly important in those regions where climate change is extending the desertification process. The rapid spread of these plants and their adaptation to hydric stress may explain this trend (Mas et al., 2015).

This paper presents the results of skin prick tests performed on patients with respiratory allergy symptoms conducted in the cities of Almería and Murcia, together with the aerobiological characteristics of the taxon *Amaranthaceae* in the cities of Almería, Cartagena and Murcia.

The study focuses on the aerobiology of the taxon *Amaranthaceae* in the three cities and the importance of summer/autumn flowering in the geographical area under study.

2. Methods

Skin prick tests were performed with the following pollen extracts: *Olea*, *Chenopodium* (*Ch. album*), *Salsola* (*S. kali*), Poaceae, Cupressaceae, *Artemisia*, *Parietaria*, and *Platanus* (Stallergenes, ALK-Abelló), on patients with respiratory allergy symptoms, referred for an allergy consultation for the first time. In Almería, these included patients referred to the clinic of Dr. Zapata Yébenes between January 2010 and December 2014. In the Region of Murcia, the sample included patients referred for an allergy consultation to the Reina Sofía General Hospital, in the city of Murcia, between January 2013 and April 2015. This hospital is the reference centre for Health Area VII (which covers the areas of Beniel, Llano de Brujas, Murcia-El Carmen, Murcia-Alquerías, Murcia-Beniján, Murcia-Infante, Murcia-Monteagudo, Murcia-Puente Tocinos, Murcia-Vistabella and Santomera), which covers a population of 202,000 people.

In relation to the *Amaranthaceae* family, the aim of this paper, the pollen extracts for *Chenopodium album* and *Salsola kali* included the allergens Che a 1, Che a 2 and Che a 3; Sal k 1, Sal k 2, Sal k 3, Sal k 4 and Sal k 5, respectively.

As regards meteorological data, temperature and rainfall in the three cities was provided by the Spanish Meteorological Agency (AEMET).

Aerobiological data were obtained in the city of Almería and from the Region of Murcia Aerobiological Network by means of three Hirst-type volumetric samplers with a suction volume of 10 L/min, located in the cities of Almería (coordinates: 36° 50' N 2° 52' W), Cartagena (37° 36' N 0° 58' W) and Murcia (37° 59' N 1° 7' W), during the period 2010–2014. The device has a drum which rotates at 2 mm/h over Melinex® tape coated with a silicone-based adhesive onto which particles ranging between 1 and 100 µm adhere (Galán et al., 2007). This tape was sliced into 48-mm strips in the laboratory, each strip corresponding to the 24 h of each day of weekly sampling. Subsequently, each daily fraction was mounted with glycerogelatin on slides for observation under the microscope. Aerobiological counts were conducted following the methodology approved by the Spanish Aerobiology Network (Galán et al., 2007) and the Aerobiology Committee of the Spanish Society of Allergy and Clinical Immunology (Subiza, 2007).

The statistical study of aerobiological and meteorological variables was performed using the SPSS 24.0 statistical package (IBM® 2016). Data on maximum, minimum and average daily temperatures and daily rainfall were employed in the study. In addition, the variables were recoded according to meteorological stations, creating a new file

containing counts of the taxon *Amaranthaceae* and rainfall per season, including rainfall with a lag of 1 (data from the prior season), 2 (data from two seasons earlier) and 3 (data from three seasons earlier) as new variables. To ascertain whether the variables in this study fit a normal or a log-normal distribution, the Kolmogorov-Smirnov or Shapiro-Wilk tests were implemented, depending on the sample size. The Pearson correlation coefficient is provided for normal and log-normal distributions; otherwise, the Spearman correlation coefficient is given.

3. Results

It was found in the skin prick tests that (Fig. 1), out of a total of 682 patients tested in the Province of Almería during the study period, 44% showed sensitization to *Chenopodium* and 43% to *Salsola*, the second and third leading causes of sensitization after olive pollen. Thirteen (2%) of these patients were found to be monosensitized to this pollen type, being sensitized both to *Chenopodium* and *Salsola*.

Out of a total of 2190 patients in the Region of Murcia, 42% were sensitized to *Salsola* and 22% to *Chenopodium*, *Salsola* being second in importance after olive pollen. Among patients sensitized to *Amaranthaceae* in Murcia, 144 (6.6%) were monosensitized to this taxon, of which 87 (4.0%) were sensitized to *Salsola*, 29 (1.3%) to *Chenopodium*, and 28 (1.3%) to both.

In addition to published values for the average annual temperature in the series of historical records for the three cities, Table 1 shows the average annual values obtained from the data supplied by AEMET for the period 2010–2014 and the average value for the period under study. As AEMET records for Cartagena exist from 1993, the average for the period 1993–2014 is also included in the table. This table also shows the following rainfall data: average annual rainfall values published for the three cities, annual values of rainfall from 2010 to 2014, and average annual rainfall during this period. For Cartagena, the table similarly includes the average annual rainfall for the period 1993–2014.

Fig. 2 shows the daily values of counts of *Amaranthaceae*, total pollen and rainfall in the cities of Almería, Cartagena and Murcia for the period 2010–2014.

Calculating the percentage of annual *Amaranthaceae* counts versus the total annual pollen count, the average value is 8.7% for Almería, ranging from 6.4% in 2013 to 13.7% in 2011; 19.2% for Cartagena, ranging from 17.9% in 2011 to 20.9% in 2013; and 7.1% for Murcia, ranging from 5.3% in 2013 to 8.3% in 2012. Table 2 gives the semi-annual pollen counts (accumulated pollen grains of the pollen type per half year) of *Amaranthaceae* and the total semi-annual pollen count (accumulated pollen grains per half year) for the three cities in each of the years 2010 to 2014.

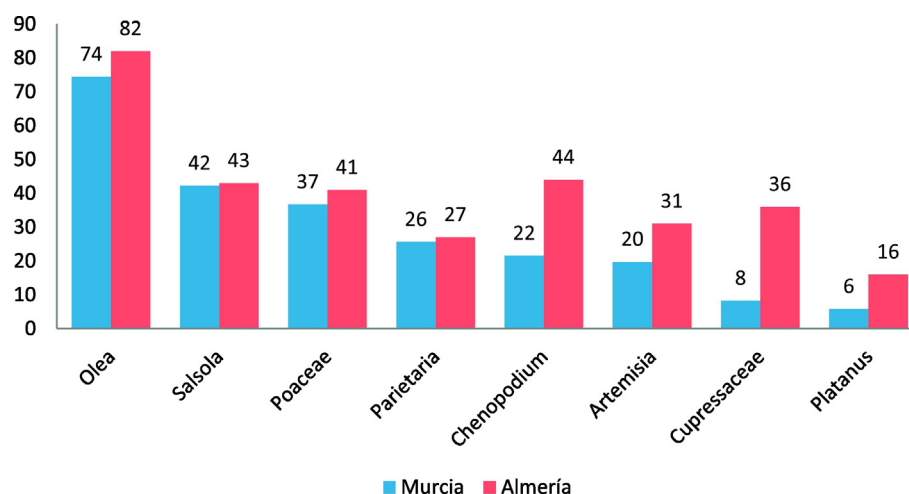


Fig. 1. Percentage of skin sensitization. Province of Almería and Region of Murcia.

Table 1

Average temperatures (°C) and annual precipitation (L/m²) in the period 2010–2014 in Almería, Cartagena and Murcia.

Year	Almería	Cartagena	Murcia
Average temperatures			
Published averages	17.9 ^a	17.3 ^b	17.6 ^c
Average AEMET data	–	18.8 (1993–2014)	–
2010	17.8	18.0	18.4
2011	18.6	19.9	19.6
2012	18.2	18.5	19.3
2013	19.1	18.8	19.3
2014	19.4	19.4	20.1
Average 2010–2014	18.6	18.8	19.3
Rainfall			
Published averages	196 ^a	231 ^b	285 ^c
Average AEMET data	–	249 (1993–2014)	–
2010	353	330	458
2011	161	195	131
2012	178	164	265
2013	139	171	231
2014	106	172	187
Average 2010–2014	187	205	255

^a El Clima en Almería, 2016. http://www.andalucia-web.net/clima_almeria.htm (accessed 19.02.16).

^b Capel Molina, J.J., 1986. El clima del territorio de Cartagena, in: Mas, J. (Ed.), Historia de Cartagena. Ediciones Mediterráneo, Murcia pp. 174 and 177.

^c González Ortiz, J.J., Sánchez Sánchez, J., 1980. La diversidad del espacio regional, in: González Ortiz, J.J. (Ed.), Geografía de la Región de Murcia. Ediciones Mediterráneo, Murcia pp. 350–351.

Fig. 3 shows the average daily counts of Amaranthaceae and total pollen counts in Cartagena for the period 2010–2014. Between days 233 and 275 (20 August and 1 October), the percentage of Amaranthaceae versus the total pollen count was 74% for Almería, 94% for Cartagena and 75% for Murcia.

The variables checked in the statistical study do not fit a normal distribution. Some of them do fit this pattern, however, after applying logarithms, though this is not the case for the pollen counts of the taxon Amaranthaceae. Consequently, the Spearman correlation coefficient was employed, showing (Table 3A):

- A positive and significant correlation at the 99% confidence level for Almería, Murcia and Cartagena between concentrations of Amaranthaceae pollen and maximum, minimum and average daily temperatures;
- A negative and significant correlation at the 99% confidence level in all three cities with rainfall.

On studying the correlations between concentrations of Amaranthaceae and rainfall by meteorological stations (Table 3B), a negative correlation is found for the three cities with rainfall in the same season, although the sign changes to positive for rainfall data from prior seasons, with variable levels of statistical significance:

- No statistically significant correlation was found for Murcia.
- Statistically significant correlations were found for Cartagena and Almería. In the case of Almería, it reached the 99% confidence level, for data from two seasons earlier. In Cartagena, the correlation between Amaranthaceae and rainfall data from two seasons earlier was significant at the 95% confidence level.

However, when this correlation is performed for each meteorological station and rainfall data from previous seasons, it can be seen that the concentration of Amaranthaceae in the city of Cartagena (Table 3C) in summer has a positive and significant correlation at the 95% confidence level with the rainfall recorded in spring and winter. This correlation is not found to be statistically significant for any season or rainfall in the other cities.

Fig. 4 shows the annual pollen counts for Amaranthaceae in each of the cities and the trend. This analysis was repeated for the values of each flowering and rainfalls, finding negative trends in all cases.

4. Discussion

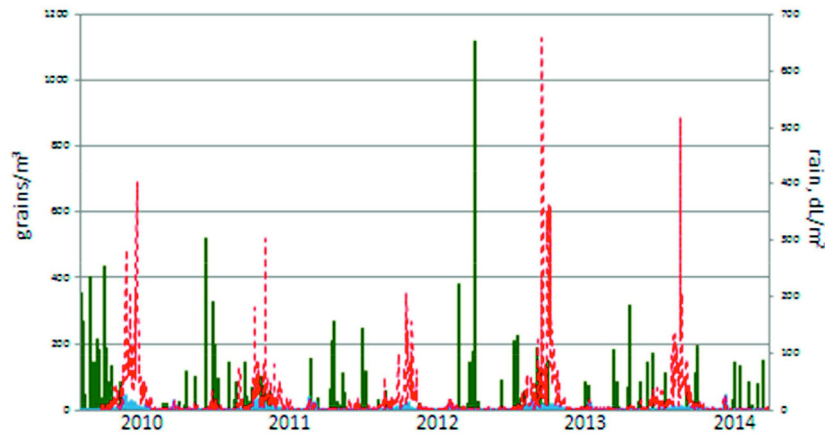
Skin sensitization in the sample of patients, both in the Province of Almería and the Region of Murcia, shows Amaranthaceae pollen to be second in importance after olive pollen. There is clearly greater sensitization to *Salsola* than to *Chenopodium* in the Region of Murcia, the results being similar for Almería. In a previous study carried out in the Region of Murcia, it was found that 61% of patients with hay fever were sensitized to Amaranthaceae, following 63% of patients sensitized to Oleaceae (Belchí-Hernández et al., 2001). In Toledo, sensitization figures were 50.3% for *Chenopodium album* L. and 44.3% for *Salsola kali* L. (Moral de Gregorio et al., 2008). In Zaragoza, figures of 42% for *Chenopodium* and 35% for *Salsola* were reported (Pola et al., 1998). The taxon Amaranthaceae is among the 6 leading causes of allergies in Spain, with 5% sensitizations in Santander (Pola et al., 2009), 8% in Córdoba (Galán et al., 1989), 50% in Zaragoza, 52% in Ciudad Real, and 58% in Toledo (Pola et al., 2009), with an average prevalence of 31%. In general, sensitizations between *Chenopodium* and *Salsola* are greater than or equal to those to *Chenopodium* (Pola et al., 2009), as found in Almería. Only in Madrid does the opposite occur, with 35% of positive skin reactions to *Salsola* compared to 30% to *Chenopodium* (Pola et al., 2009). These figures are lower than those found in Murcia, where *Salsola* accounts for up to 45% of sensitizations and *Chenopodium*, approximately 22%. These findings highlight the importance of the taxon Amaranthaceae, especially throughout Southeastern Spain. Besides, we believe its importance will grow due to the persistent drought the area is suffering and may further increase due to foreseeable climate scenarios, as suggested by other authors (Cariñanos et al., 2014; Schmidt, 2016; Ziska et al., 2009).

As to those patients monosensitized to the taxon Amaranthaceae, these comprise between 0 and 5%, depending on the area (Pola et al., 2009). The percentage of monosensitization in Almería fluctuates around the reported value (2.0%). In Murcia, however, monosensitizations exceed 6.5%. The higher percentage of monosensitization to *Salsola* (close to 4%) than to *Chenopodium* is worth noting. This percentage of monosensitization in Murcia is greater than that obtained in previous studies, namely 1.7% (Belchí-Hernández et al., 2001). Also noteworthy is the fact that monosensitized patients in Almería presented positive skin prick tests to both *Salsola* and *Chenopodium*, whereas patients monosensitized to one or the other genus coexist in the Region of Murcia, with only 1.3% being sensitized to both.

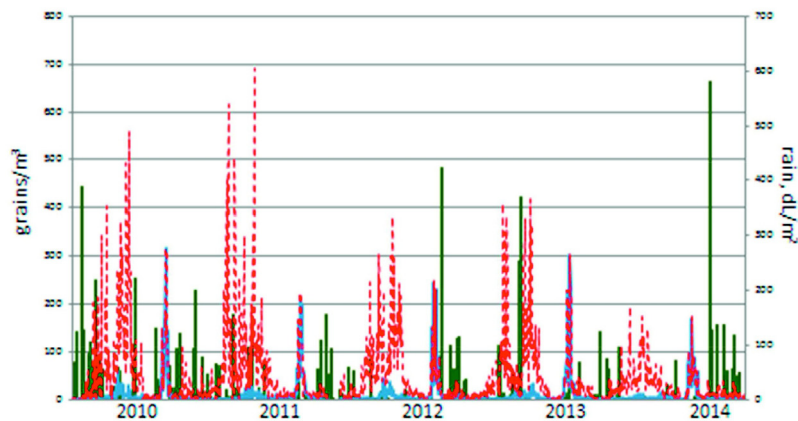
In all three cities, the average temperature during the period under study (2010–2014) was higher than the average values of historical records (Table 1). This was not the case for the period for which we have data for Cartagena from the same AEMET station (1993–2014), in which the average temperature coincides with that of the period 2010–2014. A number of publications (Fernández-Montes and Rodrigo, 2015; Gonzalez-Hidalgo et al., 2015) report this pattern, both for Spain in general and for the Southeast of the Iberian Peninsula in particular, where it is statistically significant at the 95% confidence level for the studied areas (Fernández-Montes and Rodrigo, 2015; Gonzalez-Hidalgo et al., 2015).

As for rainfall, all the data we managed show lower average annual values in the study period than historical records, all of which were below the 300-mm isohyet, corresponding to arid zones (Table 1). In fact, during the period under study, annual rainfall above 300 mm was only recorded in the three cities in 2010. This decrease in rainfall has also been previously demonstrated (Del Río et al., 2011; Fernández-Montes and Rodrigo, 2015). In these studies, the decrease in rainfall in summer was found to be statistically significant.

A) Almería



B) Cartagena



C) Murcia

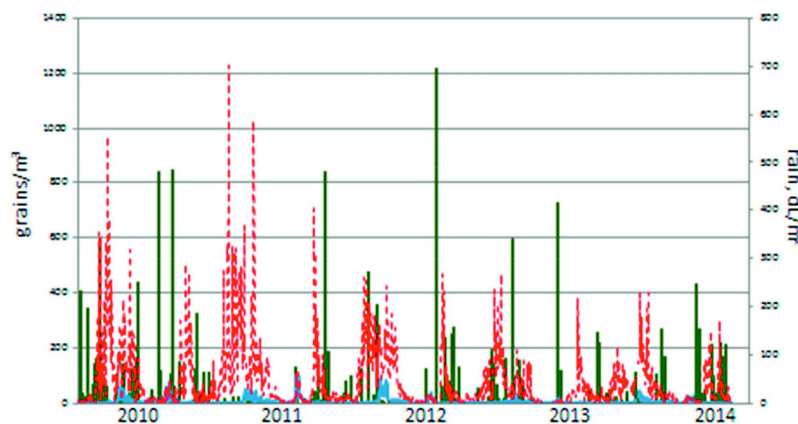


Fig. 2. Daily Amaranthaceae (grains/m³, blue line) and total pollen counts (grains/m³, red dotted line) in the period 2010–2014 and daily rainfall (dL/m², green bars). A) Almería B) Cartagena C) Murcia. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Of the three cities, Almería is the one with the lowest pollen counts and the lowest annual values for Amaranthaceae pollen, although the percentage with respect to the total is somewhat higher than for Murcia. The city with the highest pollen counts is Murcia. However, as regards Amaranthaceae pollen, the city where this pollen type is found in the highest percentages is Cartagena. As shown in Figs. 2 and 3, although different taxa of Amaranthaceae pollinate for most of the

year (Fernández-Illescas et al., 2010), there are two times of the year when levels are higher, one in spring and the other in summer/autumn, coinciding with the onset of symptoms in the population.

In Almería, the first flowering is more important in quantitative terms than the second (Table 2). However, the peak day may occur either in the first flowering (2010) or in the second (in the remaining years, although the peak day in 2013 presents the same amount in

Table 2
Peak date, peak count, counts for each flowering of Amaranthaceae, total pollen counts in the same period and % Amaranthaceae (Amar) versus total pollen counts in the three cities.

First half of the year						Second half of the year				
	Peak date	Peak date count grains/m ³	Amar flowering, grains/m ³	Total, grains/m ³	% Amar versus TPC	Peak date	Peak date count grains/m ³	Amar flowering, grains/m ³	Total, grains/m ³	% Amar versus TPC
Almería										
2010	3 May	44	834	11,465	7.27	8 Sept	27	231	963	23.98
2011	19 Apr	32	584	5943	9.82	2 Sept	39	353	908	38.87
2012	20 May	20	371	5965	6.21	7 Sept	26	313	739	42.35
2013	13 Apr	32	609	13,091	4.65	11 Sept	32	279	829	33.65
2014	6 June	13	415	8084	5.13	7 Sept	42	239	761	31.40
Cartagena										
2010	4 May	58	906	14,715	6.16	11 Sept	315	2813	4587	61.33
2011	29 Apr	35	744	14,558	5.11	12 Sept	213	2496	3519	70.93
2012	24 Apr	44	775	13,359	5.80	6 Sept	246	2327	3615	64.37
2013	1 June	32	786	12,944	6.07	11 Sept	301	2806	4240	66.18
2014	15 June	14	391	7155	5.46	9 Sept	171	1629	2985	54.57
Murcia										
2010	29 Apr	60	1004	23,984	4.19	16 Sept	74	1086	9145	11.88
2011	27 Apr	54	1507	29,100	5.18	4 Sept	102	1249	9132	13.68
2012	26 Apr	87	1680	21,855	7.69	11 Sept	37	644	6237	10.33
2013	8 May	32	658	13,337	4.93	5 Sept	17	320	4962	6.45
2014	19 Apr	46	848	12,436	6.82	12 Sept	27	602	5908	10.19

both the first and second flowering). Even so, the percentage of this pollen with respect to the total pollen count is greater in the second flowering, exceeding 20% in each year. In the first flowering, the peak day occurs between 13 April and 6 June. The peak day of the second flowering occurs within a narrower period, between 2 and 11 September.

Contrary to what occurs in the other two cities, the second peak in Cartagena is much more pronounced than the first, when the pollen counts between 20 August and 1 October reaches over 90% of the total pollen count. The peak day always occurs in the second flowering. The maximum peak of the first flowering occurs between 24 April and 15 June, that of the second in a narrow time frame, as in the case of Almería, namely between 6 and 12 September. During the second peak in

Cartagena, Amaranthaceae is practically the only pollen type in the atmosphere, so it will have a very significant impact on the health of sensitized individuals. In all three cities, the importance of this taxon in the summer/autumn is noteworthy, with the most sensitized patients to Amaranthaceae usually presenting symptoms at this time of year.

In Murcia, the first major flowering is also more important in quantitative terms than the second, the peak day being observed in the second flowering for the years 2010 and 2011. As in Almería, the percentage with respect to the total pollen count is higher for the second peak than for the first, although its relative importance in both cases is lower than in Cartagena. The appearance of the maximum peak in the first flowering occurs between 19 April and 8 May, with

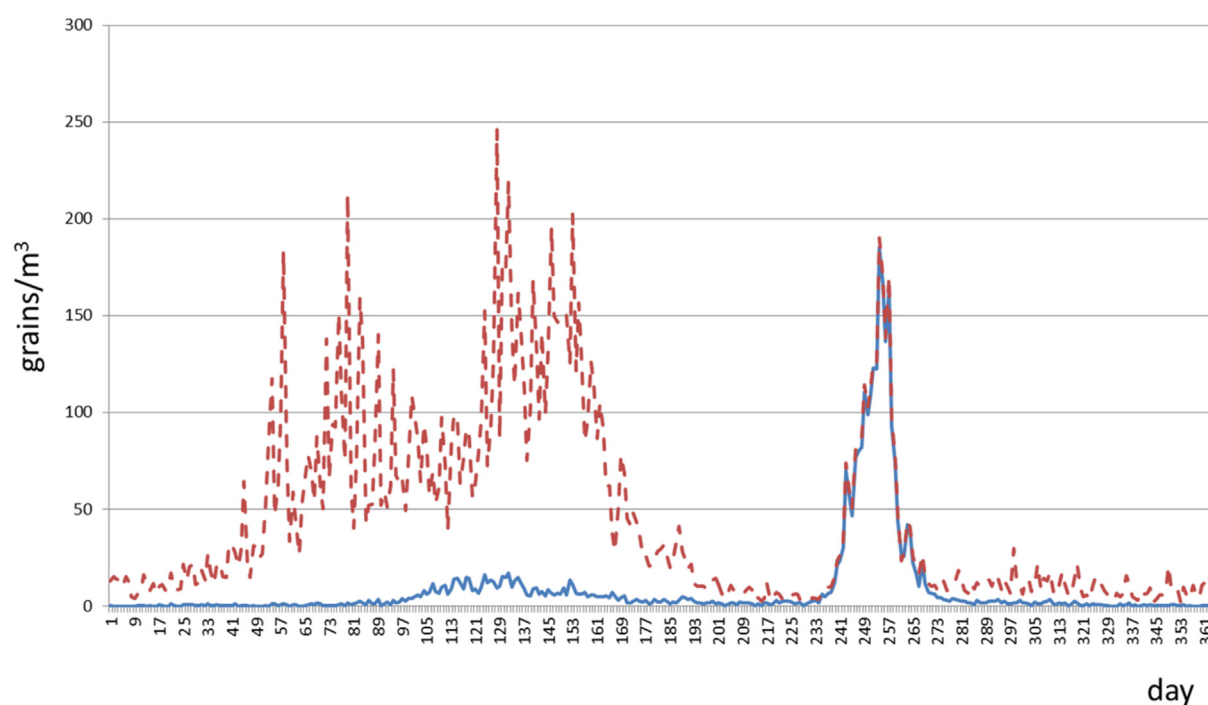


Fig. 3. Average daily Amaranthaceae (solid blue line) and total pollen (discontinuous red line) counts in the period 2010–2014, grains/m³. Cartagena. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Spearman correlation coefficients: A) versus *Amaranthaceae* pollen counts (APC) and the maximum (Tmax), minimum (Tmin) and average (Tmean) temperature. B) versus *Amaranthaceae* pollen counts and rainfall by meteorological station and data from rainfall for prior stations. APC_S: *Amaranthaceae* pollen counts in a station; Rainfall_S: rainfall in the same station of APC_S; Rainfall_S1 rainfall in the prior station of APC_S; Rainfall_S2: rainfall in two seasons earlier to APC_S. C) versus *Amaranthaceae* pollen counts (APC_S) in a station and rainfall in the prior station and in two seasons earlier.

A)				
APC	Tmax	Tmin	Tmean	Rainfall
Almería	0.364**	0.381**	0.323**	−0.164**
Cartagena	0.508**	0.492**	0.506**	−0.152**
Murcia	0.516**	0.477**	0.512**	−0.099**
**Significance level 99%, p-value < 0.01.				
B)				
APC_S	Rainfall_S	Rainfall_S1	Rainfall_S2	
Almería	−0.363	0.339	0.713**	
Cartagena	−0.410	0.007	0.510*	
Murcia	−0.229	0.035	0.346	
*Significance level 95%, p-value < 0.05; **Significance level 99%, p-value < 0.01.				
C)				
Cartagena ^a				
APC_S	Rainfall_S1	Rainfall_S2		
Spring	0.600	−0.100		
Summer	0.900*	0.900*		
Autumn	−0.400	−0.100		
Winter	0.359	−0.667		
*Significance level 95%, p-value < 0.05; ^a Almería and Murcia are not shown as no significant correlation was found for these cities.				

the second flowering once again occurring within a narrow time frame, namely between 4 and 16 September.

The majority of published studies on this pollen type do not include separate analyses of the two flowerings. Moreover, this taxon is reported to be present in the air for a longer period, ranging from spring to autumn, despite the finding that the maximum peak day occurs in spring or summer. In Palma de Mallorca, for example, the peak occurred prior to 20 May in the period 2004–2009 and on 29 August in 2010 (Boi and Llorens, 2013). In Salamanca, it occurred on 17 June 2000 and after 24 July from 2001 to 2007, transpiring on 8 September in 2004 (Rodríguez de la Cruz et al., 2012). Only the paper by Fernández-Illescas et al. (2010) reports two peaks in the aerobiological data of this pollen type in a study conducted in the marshes of Huelva. In 2006 and 2007, these authors found maximum peak values of 36 and

19 grains/m³ for the first flowering and 62 and 49 grains/m³ for the second.

The literature reports lower values of concentrations of this taxon than those present in the cities of Almería, Cartagena and Murcia. For example, maximum values of 100 grains/m³ air were recorded in Málaga and Almería (Recio et al., 2006), a concentration greatly surpassed in the bioaerosol concentration in Cartagena in the summer/autumn flowering. Peak day maximums of 31 grains/m³ have been reported in Palma de Mallorca (Boi and Llorens, 2013) and 22 grains/m³ in Salamanca, with annual counts ranging between 64 pollen grains/m³ in 2000 and 474 grains/m³ in 2005 (Rodríguez de la Cruz et al., 2012).

Moreover, the aforementioned studies highlight the low percentage of this taxon with respect to the total annual pollen count: 1.6% in a multicentre study in Spain (Pola et al., 2009), 1.5% in Castile-La Mancha, except in Albacete, where values above 5% of the total pollen count were recorded (Pérez-Badia, 2015), the same percentage as that reported for Zaragoza (Pola et al., 1998). Despite the high degree of skin sensitization, with a significant incidence of polysensitized patients, it should be recalled that monosensitizations of between 0 and 5% have been reported depending on the area (Pola et al., 1998).

In view of the results presented and discussed above, we believe that to assess the aerobiological importance of this taxon properly, the analysis should not be performed on the annual total count. Rather, considering the pattern presented in Figs. 2 and 3 and Table 2, the periods corresponding to the two upsurges in concentrations should be analyzed separately. In general, the first peak shows a smaller percentage with respect to the total pollen count, sharing the period with many other types of spring flowering pollen and contributing to the total allergen load in the bioaerosol. Its presence is important, nonetheless, given the high incidence of polysensitized patients and the possibility of cross-reactivity (Villalba et al., 2014).

However, the prevalence of this taxon in the summer/autumn period is indisputable in this geographical area. This peak represents >10% with respect to the total pollen count in all three cities. Furthermore, when this analysis is performed for the specific period corresponding to this peak, between 20 August and 1 October, the percentages rise above 70%, especially in the city of Cartagena, where a value of 94% of the total pollen count is reached. We thus believe that, in a patient presenting positive skin prick tests to *Amaranthaceae* who does not have symptoms in late summer, the reaction is more likely due to cross-reactivity or subclinical sensitization, rather than to major sensitization to this pollen type. Those patients who are actually sensitized to

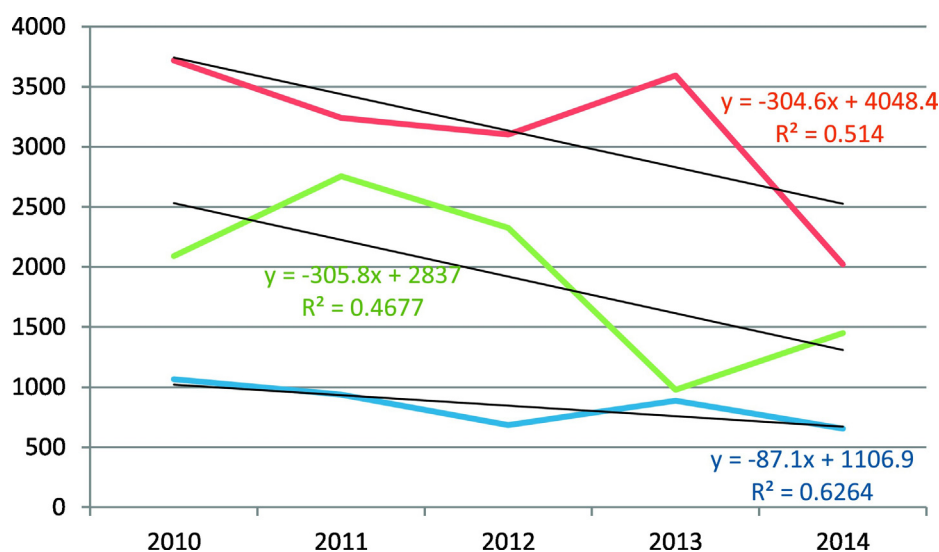


Fig. 4. Annual *Amaranthaceae* pollen counts in Almería, Cartagena and Murcia in the period 2010–2014 and trend (Almería, blue; Cartagena, red; Murcia, green) at the three sampling points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Amaranthaceae will present symptoms in the summer/autumn period due to its prevalence in the area under study.

It is markedly present in both periods, as polysensitizations are very common in the study area. Hence, all the taxa present in the atmosphere should be known in order to ensure proper diagnosis and treatment.

In all three cities, a decrease in Amaranthaceae pollen counts was recorded during the period under study for the flowering in both the first part of the year and in the second. The slope of the trend line is almost the same for Cartagena and Murcia, being much lower than for the city of Almería. We believe that this decrease in concentrations is directly related to the low rainfall recorded in the study area during the period 2010–2014. Adverse conditions give rise to physiological strategies that lead to minimizing reproductive functions to a degree still compatible with life, adapting to the availability of water, hydric stress being one of the factors affecting the normal development of plants. Such a situation results in a substantial reduction in pollen counts (Cariñanos et al., 2004).

Analysis of the correlation between the concentrations of the taxon Amaranthaceae and maximum, minimum and average daily temperatures shows a positive relationship between these variables. That is, an increase in temperature tends to increase the Amaranthaceae pollen count in the atmosphere, a pattern which has been reported in other studies (Emberlin, 1994; García-Mozo et al., 2006; Moreno-Grau et al., 2000; Rodríguez de la Cruz et al., 2012), being statistically significant at the 99% confidence level in all three cities. The pattern as regards rainfall also coincides with that reported by various authors (Cariñanos et al., 2004; Moreno-Grau et al., 2000), where it is explained as the washing effect of rain, leading to a decrease in the concentrations of airborne pollen. However, this correlation is not usually found to be statistically significant, though it was found to be so in the three cities.

The second effect of rainfall is to increase the plant biomass, producing an increase in lagged pollen levels over time. This effect is harder to evidence as the correlation is not usually statistically significant. In order to verify this effect, the Amaranthaceae and rainfall variables were grouped by meteorological stations, finding a negative correlation with rainfall at the same station, but a positive correlation with rainfall data from previous seasons. The correlation was statistically significant at the 95% confidence level with rainfall data from the two previous seasons for Cartagena, and at the 99% confidence level with two seasons earlier in Almería, thus showing the favourable influence of prior rainfall on pollen levels (García-Mozo et al., 2006). A positive effect of rainfall is observed in Cartagena for the concentration of Amaranthaceae in summer and recorded rainfall during the previous spring and winter, being statistically significant at the 95% confidence level.

Some authors have already highlighted the possibility that the modification of environmental conditions, with changes in the patterns of rainfall distribution and rising temperatures, results in an increase and expansion of xerophyte plant species and individuals (Fernández-González et al., 2005), which include some of the species belonging to the Amaranthaceae family (Cariñanos et al., 2014). Thus, allergies to Amaranthaceae pollen may become prominent in other geographical areas, approaching the data found in the South-east of Spain.

5. Conclusions

Amaranthaceae pollen is the second leading cause of hay fever in the Southeast of the Iberian Peninsula. Its aerobiology shows the importance of spring flowering due to its contribution to the overall allergen load in the bioaerosol. However, it is especially prevalent in summer/autumn, a period in which it is found practically alone in the bioaerosol or at significant proportions of above 70%, depending on the specific area, such as in Cartagena. The situation of persistent drought in the area during the period under study justifies the quantitative decrease produced in aerobiological records of this taxon.

The foreseeable increase and expansion of Amaranthaceae makes the data from Southeastern Spain a benchmark with respect to the aerobiology of this type of pollen.

Acknowledgements and funding

The authors wish to express their thanks to Ms. Paula García López, Region of Murcia Aerobiological Network technician; the Fundación para la Formación e Investigación Sanitarias de la Región de Murcia (FFIS), which funded the Network technician's contract from 2013 to 2014; the Allergy and Clinical Immunology Service, Reina Sofía Hospital, Murcia, for the taking of samples carried out in that city; and AEMET, for providing meteorological data. Anonymous referees are also thanked for contributing to improving the quality of the paper.

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