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Chapter 16

AEROBIOLOGICAL NOTES OF CHENOPODIACEAE-AMARANTHACEAE POLLEN IN THE MIDDLE-WEST OF SPAIN

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ABSTRACT

The aim of the present chapter was to describe the Chenopodiaceae-Amaranthaceae pollen dynamics in the atmosphere of two cities of the Middle-West of Spain (Salamanca and Valladolid). Samples were collected by the volumetric method with the aid of two Burkard spore-traps located in the centre of both urban cities during years 2005 and 2006. This pollen type was mainly detected in the atmosphere between late Spring and late Summer, with a Main Pollen Season registered between late May and early October and maximum concentrations detected in August. The intra-diurnal pattern, calculated by means of three different methods, was very similar for both towns reaching a higher hourly concentration percentage in the second half of the day. The correlations obtained between daily pollen counts and different meteorological parameters showed that the airborne presence of this pollen type is positively associated with temperature and negatively with rainfall during MPS. According to known threshold (10-15 pollen/m^3), Chenopodiaceae-Amaranthaceae pollen concentrations exceeded this threshold during 1 day in 2005 and during 12 days in 2006.

INTRODUCTION

The Chenopodiaceae and Amaranthaceae families are widespread worldwide and make a stenopalynologic group featuring rather similar pollen grains [1]. Pollen grains of both families are present in the atmosphere of many cities in Africa [2], America [3], Asia [4] and Europe [5]. Many members of these families have been involved in inducing pollinosis [6], playing the orbicules located in the pollen exine an important role as possible important vectors of allergens [7]. Various authors have reported a high degree of cross-reactivity among related pollens of different species belonging to those families [8] and even among some foods, such as asparagus, garlic and onion [9]. In that sense, the relevance of panallergens polcalcin and profilin in pollen-pollen cross-sensitization, is important, due to this family of proteins presents highly conserved amino acid sequences [10]. One of the allergens, Che a 3, displays similarity with polcalcins from other pollen types of other genus such as *Olea* or *Betula* [11]. Therefore, polcalcin and profilin could be involved in cross-reactivity with other pollen sources, explaining the highly frequent polysensitization of patients allergic to the Chenopodiaceae-Amarathaceae pollen [12]. In Spain, the prevalence of sensitization is between 30% and 40% in patients with hay fever symptoms [13], especially in Summer [14].

This chapter attempted to determine the aerobiological patterns of the Chenopodiaceae-Amaranthaceae pollen in the atmosphere of two cities located in the Middle-West of Spain (Salamanca and Valladolid) and to evaluate the influence of meteorological parameters in its airborne distribution. In addition, the importance of regional studies in order to a better knowledge of the daily and seasonal airborne pollen character is increasing nowadays [15], and given the fact that allergologists could establish the main season of acute sensitivity to prevent pollinosis.

MATERIAL AND METHODS

Aerobiological samples were collected during years 2005 and 2006 bye means of two Hirst type-traps, Burkard model (Burkard Manufacturing Co., Rickmansworth, Hertfordshire, UK), one located at the top of a central historical building placed in Salamanca and another sited at the roof of the Río Hortega University Hospital of Valladolid. In both cases, they were placed at a height of 20 m above the ground level. Sampling method, slide preparation, and data interpretation were performed according to the Spanish Aerobiology Network [16].

Salamanca (40° 58′N, 5° 40′W; population 155,740) and Valladolid (41° 39′N, 4° 44′W; population 318,461) are located in the Castilla-León region at a height of 800 and 691 m above the sea level, respectively. Salamanca is surrounded by wide expanses of Mediterranean pastures, whereas the vegetation surroundings of Valladolid are composed of arable farming lands. Both cities have a continental climate [17], characterised by cool winters, warm summers and a low annual rainfall level, which determines a drought season during the summer period.

A 5-day running mean was calculated for both cities and years and plotted to assess the seasonal trend. For each year, the Main Pollen Season (MPS) was determined by taking 95% of the annual total recorded pollen concentration using cumulative values [18]. Starting and

ending dates were established corresponding to the days when the sum of the daily concentrations reaches 2,5% of the total sum and when the sum reaches 97,5%, respectively. The pre-peak period (PRE) includes the period between the starting date of the MPS and the date on which the highest concentration is reached. The post-peak (POST) comprises the period from the date of the peak value until the ending date of the MPS.

The Spearman's correlation coefficients between daily pollen concentration (expressed as pollen/m^3) and some daily meteorological parameters (temperature, precipitation, relative humidity, wind speed, winds from the first, second, third and forth quadrant, frequency of calms and total hours of sunshine) were analyzed for each year and city during the MPS, PRE and POST periods. The non-parametric Spearman's coefficient was chosen because daily pollen counts are not normally distributed. Meteorological data were supplied by the "Agencia Estatal de Meteorología" (AEMET-Spanish State Meteorological Agency), whose meteorological stations Salamanca-Matacán and Valladolid-Observatorio are located 10 and 5 km east from the pollen sampling sites, respectively. Statistical analysis was carried out by SPSS (v.12) software package.

In addition, the intra-diurnal variations recorded during the MPS of the monitored period have been studied, taking into account three different models described by some authors [19]. In the first model, the value registered in each hour is represented by the average of the values of that hour, while in the second model, an ideal day was calculated by dividing the sum of the values of each hour by the number of days in which Chenopodiacae-Amaranthaceae pollen was present. In both cases, the values for each hour are expressed as a percentage of the daily total. In the third model, it is important whether rainfall occurred or not. The daily average of the pollen type was calculated using the total number of days of the MPS period as the denominator. After that, we selected just the dry days with a daily value equal to or higher than the average previously calculated. In this model, the value obtained for each hour is represented as described in the first model, with the aim to compare the three models. For the graphical representation we used a 3-hour running mean to smooth the tendency.

RESULTS AND DISCUSSION

There were not significant differences in meteorological parameters data between Salamanca and Valladolid during the studied period, except for the increment of rainfall in year 2006, especially at Valladolid, and the lower values for frequency of calm registered in Valladolid (Table 1). The annual pollen index of Chenopodiaceae-Amaranthaceae pollen grains was very similar in the atmosphere of Salamanca and Valladolid during 2005 (248 and 211) and 2006 (474 and 418, respectively). The differences in annual pollen sums could be related to rainfall occurred between June and August for the two cities during year 2006, because of its positive effect on the development of the herbal species, and the lower values of rainfall registered in these two cities during year 2005 (Fig. 1), both related with data corresponding with reference period (1971-2000).

Table 1. Meteorological parameters values registered along the monitored period at both studied localities (Salamanca and Valladolid).

	Tmean	Tmax	Tmin	R	RH	WS	NE	SE	SW	NW	CF	Sunshine
Salamanca												
2005	11.6	18.3	5.4	278	66	11	32.8	15.7	24.9	17.6	8.3	7.8
2006	12.5	18.8	6.7	347	69	11	23.6	14.3	23.9	15.0	6.5	7.3
Valladolid												
2005	12.4	19.0	6.6	275	62	10	37.4	10.7	26.1	22.8	0.5	8.2
2006	13.1	19.4	7.6	523	68	9	32.5	13.0	29.1	23.9	0.4	7.3

Tmean mean daily average temperature (ºC). **Tmax** maximum daily average temperature (ºC). **Tmin** minimum daily average temperature (ºC). **R** total daily rainfall (mm). **RH** daily average relative humidity (%). **WS** daily average wind speed (km/h). **Wind NE** daily average frequency of north-easterly winds (%). **Wind SE** south-easterly winds (%). **Wind SW** south-westerly winds (%). **Wind NW** north-westerly winds (%). **CF** daily average frequency of calms (%). **Sunshine** daily average sunshine (hours).

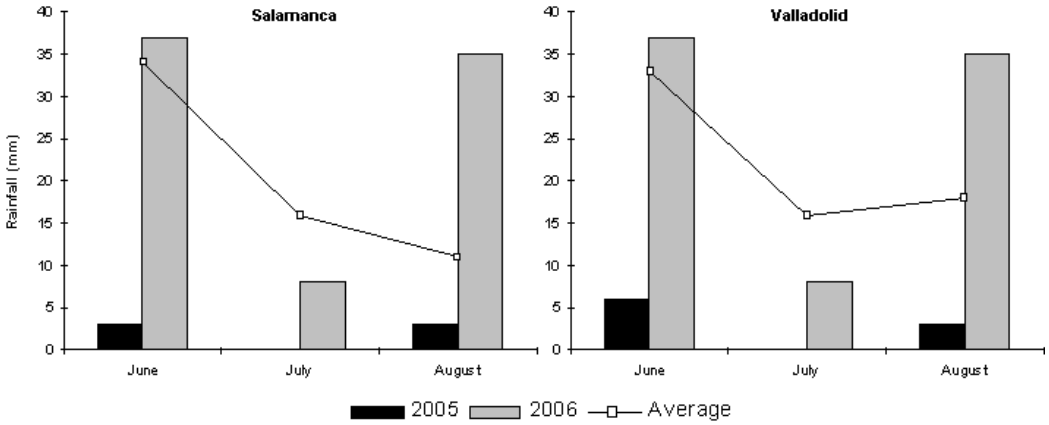

Figure 1. Total monthly rainfall in Salamanca and Valladolid during years 2005, 2006 and average period 1971-2000.

Seasonal variations in the average of the daily pollen concentrations for the studied period are analyzed in Fig. 2. The airborne presence of this family already started at early April. From late May, the daily levels of pollen increased gradually until mid-late August, when they reached the highest peak daily values (Table 2), ranging from 10 pollen grains per cubic meter of air (p/m^3) on 15th August 2005 in Valladolid to 22 p/m^3 on 9th August 2006 in Salamanca. Subsequently, the mean counts decreased until early November. Main Pollen Season (MPS) came from May to October and its length was quite similar in 2005 (Table 2), but different in 2006, because of the smaller length of MPS in Valladolid (107 days) than Salamanca (139). The onset of MPS was earlier in Salamanca than in Valladolid (Table 2), at least two weeks in the two-years studied, whereas the end date did not show significant differences in Salamanca. In the city of Valladolid, this end date had an irregular pattern in the studied period, being later in year 2005 and earlier in year 2006 than the end date obtained for Salamanca. MPS of this pollen type and annual pollen indexes in Salamanca and

Valladolid correspond to those already described in Madrid [20] and Cáceres [21], also located in the Middle of Spain, being longer and higher, respectively, than other northern Spanish cities [22,23]. However, other sampling points placed in the South of Spain, such as Murcia [24] and Almería [25] displayed a longer MPS and a higher annual pollen index value, due to an earlier onset and a great abundance of Mediterranean "maquis" in the vegetation surroundings [26], where species from Chenopodiaceae predominate.

Table 2. Seasonal behaviour of airborne Chenopodiaceae/Amaranthaceae pollen.

Chenopodiaceae/ Amaranthaceae	Annual Pollen Index	Start MPS	End MPS	Length MPS	Peak day	Prepeak length	Peak day (p/m^3)
			Salamanca				
2005	248	8-May	9-Oct	155	28-Aug	112	12
2006	474	27-May	12-Oct	139	9-Aug	75	22
			Valladolid				
2005	211	26-May	28-Oct	156	15-Aug	82	10
2006	418	18-Jun	2-Oct	107	10-Aug	54	14

MPS Main Pollen Season

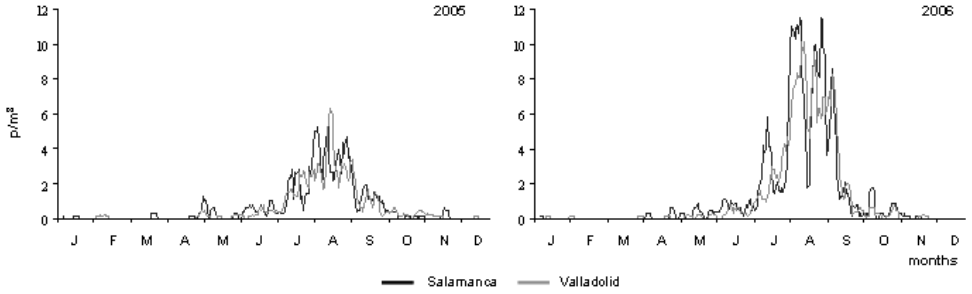

Figure 2. Seasonal variation of Chenopodiaceae-Amaranthaceae pollen during years 2005 and 2006 in both studied cities (5-days running means).

A study of the intra-daily variation (Fig. 3) revealed that there were similarities between both cities' behaviour, because pollen start to accumulate after 9 AM and the highest values were recorded during the central hours of the day, specifically between 10 AM and 7 PM. This pattern is similar to that reported for Cáceres [27], Málaga [28], or even for other cities in America, such as La Plata [29], and confirmes the occurrence of anthesis and pollen dispersion during daylight [30].

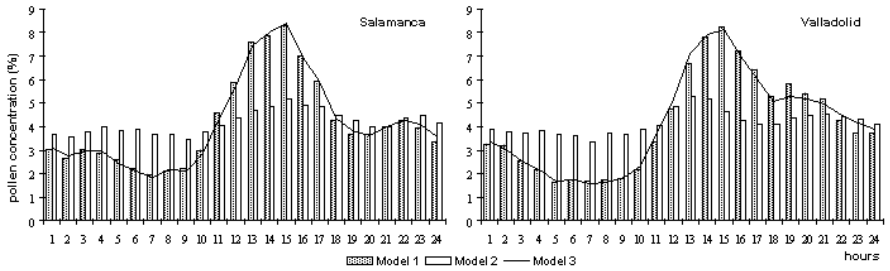

Figure 3. Chenopodiaceae-Amaranthaceae intra-diurnal patterns (3-h running mean).

Taking into account the relationship between meteorological parameters and the airborne pollen concentration (Table 3), temperature had a positive and significant correlation with pollen concentrations registered during the MPS, PRE and POST periods in both cities, because temperatures tends to increase the atmospheric pollen content [31], as other authors revealed for Chenopodiaceae-Amaranthaceae airborne pollen [32,33].

Table 3. Spearman´s correlation coefficients established for airborne Chenopodiaceae/Amaranthaceae pollen concentrations and meteorological parameters at both studied locations along the Main Pollen Season (MPS), Pre-peak (PRE) and Post-peak (POST) periods.

	Salamanca			Valladolid		
	MPS (n=294)	**PRE (n=187)**	**POST (n=107)**	**MPS (n=263)**	**PRE (n=136)**	**POST (n=127)**
Tmean	.335**	.335**	.641**	.399**	.290**	.739**
Tmax	.416**	.398**	.658**	.396**	.256**	.696**
Tmin	.184**	.195**	.434**	.356**	.264**	.641**
R	-.128*	-.041	-.546**	-.206**	-.175*	-.256**
RH	-.047	-.148*	.131	-.286**	-.163	-.521**
WS	.157**	.091	.051	.000	-.053	.116
Wind NE	.307**	.312**	.327**	.158*	.172*	.216*
Wind SE	.198**	.182*	.205*	-.120	-.085	-.164
Wind SW	-.251**	-.217**	-.270**	-.174**	-.160	-.230**
Wind NW	-.202**	-.312**	.003	.076	-.072	.173
CF	.051	-.040	.541**	-.019	-.009	-.059
Sunshine	-.161**	-.067	-.326**	.136*	-.093	.497**

Tmean mean daily average temperature (ºC). **Tmax** maximum daily average temperature (ºC). **Tmin** minimum daily average temperature (ºC). **R** total daily rainfall (mm). **RH** daily average relative humidity (%). **WS** daily average wind speed (km/h). **Wind NE** daily average frequency of north-easterly winds (%). **Wind SE** south-easterly winds (%). **Wind SW** south-westerly winds (%). **Wind NW** north-westerly winds (%). **CF** daily average frequency of calms (%). **Sunshine** daily average sunshine (hours).

Significance levels: *****, 95%; ******, 99% (values in grey indicate not significant correlation).

Rainfalls and relative humidity were observed to have a converse behaviour, with a negative influence, especially in Valladolid during MPS, PRE and POST periods, displaying similar results in Toledo [34] and Cartagena [35] with relative humidity, due to the known wash off effect of this meteorological parameter on pollen concentrations [36]. Wind speed showed a positive and significant influence in Salamanca MPS, as also occurred in Almería [25], whereas frequency of calms had a positive and significant correlation only during POST in Salamanca. The effect of moderate wind velocity on promoving the emission of pollen into the air has been demonstrated [37], but even after flowering period it could facilitate its airborne presence. North-easternly winds had a significant and positive effect on Chenopodiaceae-Amaranthaceae airborne pollen concentrations in both cities, conversely to south-westernly winds, being more statistically significant in Salamanca. In this city, south-easternly winds had also a positive correlation and north-westernly winds had a negative effect. In general terms, any pollen type increases its airborne levels when the wind direction is favourable to the placement of main producing species [38].

In several cases, threshold concentration of specific airborne pollen promoving allergy symptoms when exceeded [39] were established, that in the case of Chenopodiaceae-Amaranthaceae pollen was 10-15 pollen/m^3 [40]. In Salamanca and Valladolid, this pollen type exceeded its threshold value during 1 day in 2005, according with the peak days in late August, and 12 days in 2006 (Fig. 4.): 11 days in August and 1 day in September for Salamanca and 10 days in August and 2 days in September for Valladolid.

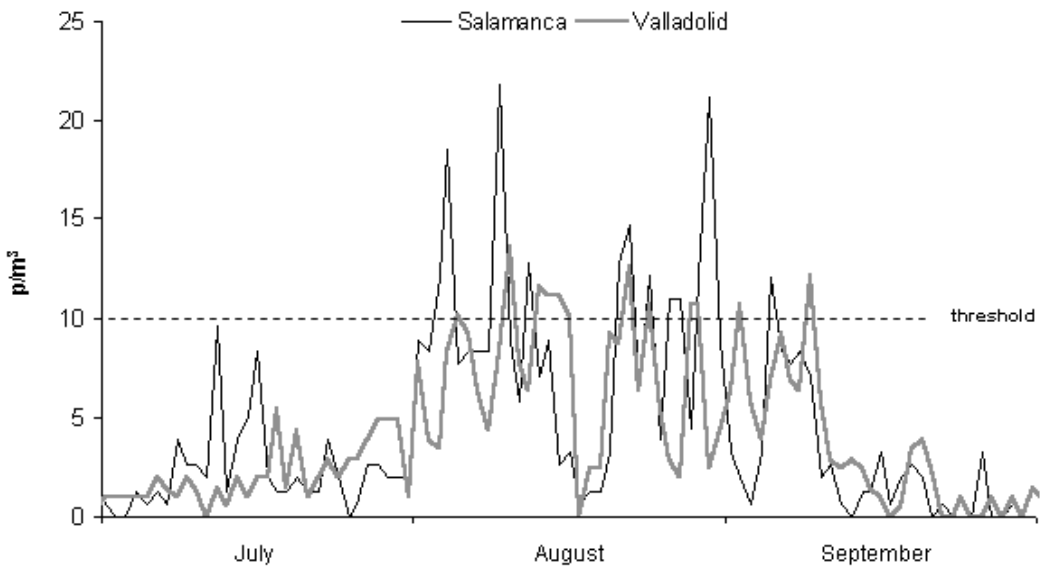

Figure 4. Seasonal variation of Chenopodiaceae-Amaranthaceae in July, August and September of year 2006, with threshold value of airborne pollen concentrations in Salamanca and Valladolid.

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REFERENCES

[1] Candau, P. (1987). *Chenopodiaceae.* In: B. Valdés, M. J. Díez & I. Fernández (Eds.), Atlas polínico de Andalucía Occidental (pp. 103-105). Sevilla, Spain: Instituto de Desarrollo regional nº43, Universidad de Sevilla. Excma. Diputación de Cádiz.

[2] Al-Qura'n, S. (2008). *Analysis of Airborne Pollen Fall in Tafileh, Jordan, 2002-2003*. World Appl Sci J, 4(5), 730-735.

[3] Rocha Estrada, A., Alvarado Vázquez, M. A., Torres Cepeda, T. E. & Foroughbakhch Pournavab, R. (2008). *Principales tipos polínicos presentes en el aire de la zona norte del área metropolitana de Monterrey, Nuevo León*. Ciencia UANL, 11(1), 69-76.

[4] Kaplan, A. (2004). *Airborne Pollen Grains in Zonguldak, Turkey, 2001-2002*. Acta Botanica Sinica, 46(6), 668-674.

[5] Weryszko-Chmielewska, E. & Piotrowska, K. (2004). *Airborne pollen calendar of Lublin, Poland.* Ann Agric Environ Med, 11, 91-97.

[6] Lombardero, M., Duffort, O. & Carreira, J.(1991). *Allergenic significance of chenop pollen.* In G. D'Amato, F.Th. M. Spieksma & S. Bonini (Eds.), Allergenic pollen and pollinosis in Europe (128-131). Oxford, United Kingdom: Blackwell Scientific Publications.

[7] Vinckier, S. & Smets, E. (2001). *The potential role of orbicules as a vector of allergens.* Allergy, 56, 1129-1136.

[8] Lombardero, M., Duffort, O., Sellés, J. G., Hernández, J. & Carreira, J. (1985). *Cross-reactivity among Chenopodiaceae and Amaranthaceae.* Ann Allergy, 54(5), 430-436.

[9] Anibarro, B., Fontela, J. L. & De la Hoz, F. (1997). *Occupational asthma induced by garlic dust.* J Allergy Clin Immunol, 100, 734-738.

[10] Barderas, R., Villalba, M., Batanero, E., Pascual, C. Y. & Rodríguez, R. (2003). *Role of profilin and polcalcin in chenopod pollen allergy.* J Allergy Clin Immunol, 111, 1132-1133.

[11] Florido, J. F., Quiralte, J., Sáenz de San Pedro, B. & Bartolomé, B. (2000). *Crossreactivity between Olea europaea and Chenopodium album pollens.* Allergy, 55 (supl 63), 158.

[12] Barderas, R., Villalba, M., Pascual, C. Y., Batanero, E. & Rodríguez, R. (2004). *Profilin (Che a 2) and polcalcin (Che a 3) are relevant allergens of Chenopodium*

album pollen: Isolation, amino acid sequences, and immunologic properties. J Allergy Clin Immunol, 113, 1192-1198.

[13] Pola, J. (2003). *Alergia a pólenes de Quenopodiáceas*. Rev Esp Alergol Inmunol Clin, 18, 39-44.

[14] Galán, C., Infante, F., Ruiz de Clavijo, E., Guerra, F., Miguel, R. & Domínguez, E. (1989). *Allergy to pollen grains from Amaranthaceae and Chenopodiaceae in Córdoba, Spain. Annual and daily variation of pollen concentration.* Ann Allergy, 63(11), 435-438.

[15] Munuera, M. (2002). *Interés de los registros aerobiológicos regionales. Originalidades aerobiológicas de la ciudad de Murcia*. Anales de Biología, 24, 185-194.

[16] Domínguez, E., Galán, C., Villamandos, F., & Infante, F. (1991). *Manejo y evaluación de los datos obtenidos en los muestreos aerobiológicos.* REA, 1, 1-18.

[17] Capel Molina, J. J. (1981). *Los climas de España*. S.A. Barcelona: Ed. Oikos-Tau.

[18] Andersen, T. (1991). *A model to predict the beginning of the pollen season*. Grana, 30, 269-274.

[19] Sánchez Reyes, E., Rodríguez de la Cruz, D., Sanchís Merino, M. E. & Sánchez Sánchez, J. (2009). *Meteorological and agricultural effects on airborne Alternaria and Cladosporium spores and clinical aspects in Valladolid (Spain).* Ann Agric Environ Med, 16, 53-61.

[20] Gutiérrez Bustillo, M., Cervigón Morales, P. & Pertiñez Izquierdo, C. (2002). *Aerobiología en Madrid: Estación de Ciudad Universitaria (2000–2001)*. Rea, 7, 225-230.

[21] Paulino, R., Tormo, R., Silva, I. & Muñoz, A. F. (2002). *Aerobiología en Extremadura: Estación de Cáceres (2000–2001).* Rea, 7, 177-182.

[22] Vega Maray, A. M., Fernández-González, D., Valencia-Barrera, R. M., Fernández Salegui, A. B., Santos, F., & Latasa, M. (2002). *Aerobiología en Castilla y León: Estación de León (2000–2001).* Rea, 7, 119-124.

[23] Rodríguez-Rajo, F. J., Jato, V. & Aira, M. J. (2003). *Pollen content in the atmosphere of Lugo (NW Spain) with reference to meteorological factors (1999-2001).* Aerobiologia, 19, 213-225.

[24] Munuera Giner, M., Carrión García, J. S. & Navarro Camacho, C. (2002). *Seasonal fluctuations of the airborne pollen spectrum in Murcia (SE Spain). Some peculiarities in the pollen callendar*. Aerobiologia, 18, 141-151.

[25] Sabariego, S., Díaz de la Guardia, C., Alba, F., Moya, C. & Cruz, S. (2003). *Estudio aerobiológico e incidencia de sensibilización del polen de Chenopodiaceae/ Amaranthaceae en Almería*. Polen, 13, 227-235.

[26] Blanco, E., Casado, M. A., Costa, M., Escribano, R., García, M., Génova, M., Gómez, F., Moreno, J. C., Morta, C., Regata, P. & Sainz, H. (1997). *Los bosques Ibéricos.* Barcelona, Spain*:* Planeta Editorial.

[27] Muñoz Rodríguez, A. F., Silva Palacios, I., Tormo Molina, R., Moreno Corchero, A. & Tavira Muñoz, J. (2000). *Dispersal of Amaranthaceae and Chenopodiaceae pollen in the atmosphere of Extremadura (SW Spain).* Grana, 39, 56-62.

[28] Recio, M., Trigo, M. M., Toro, F. J. & Cabezudo, B. (1998). *Incidencia del polen de Chenopodiaceae-Amaranthaceae en la atmósfera de Málaga y su relación con los parámetros meteorológicos.* Acta Botanica Malacitana, 23, 121-131.

[29] Nitiu, D. S. (2004). *Intradiurnal fluctuation pollen in La Plata, Argentina. Part I: Herbaceous taxa.* Aerobiologia, 20, 69–74.

[30] Latalowa, M., Uruska, A., Pedziszewska, A., Góra, M. & Dawidowska, A. (2005). *Diurnal patterns of airborne pollen concentration of the selected tree and herb taxa in Gdansk (northern Poland).* Grana, 44, 192–201.

[31] Rodríguez de la Cruz, D., Sánchez Reyes, E. & Sánchez Sánchez, J. (2008). *Aerobiological study of Fagaceae pollen in the middle-west of Spain.* Aerobiologia, 24, 67–76.

[32] González Minero, Candau Fernández-Mensaque, P., Tomás, C. & Morales, J. (1997). *Patrones de distribución de concentraciones polínicas de varios tipos de polen alergógenos (herbáceas) en relación con factores climatológicos*. Rev Esp Alergol Inmunol Clín, 12(6), 357-362.

[33] Cariñanos, P., Galán, C., Alcázar, P. & Domínguez, E. (2004). *Airborne pollen records response to climatic conditions in arid areas of the Iberian Peninsula*. Environ Exp Bot, 52, 11-22.

[34] García-Mozo, H., Pérez-Badia, R., Fernández-González, F. & Galán, C. (2006). *Airborne pollen sampling in Toledo, Central Spain*. Aerobiologia, 22, 56-66.

[35] .Moreno-Grau, S., Angosto, J. M., Elvira-Rendueles, B., Bayo, J., Moreno, J. & Moreno-Clavel, J. (2000). *Effects of meteorological parameters and plant distribution on Chenopodiaceae-Amaranthaceae, Quercus and Olea airborne pollen concentrations in the atmosphere of Cartagena (Spain).* Aerobiologia, 16, 17-20.

[36] Emberlin, J. (1994). *The effects of patterns in climate and pollen abundante on allergy*. Allergy, 49, 15-20.

[37] Damialis, A., Gioulekas, D., Lazopoulou, C., Balafoutis, C. & Vokou, D. (2005). *Transport of airborne pollen into the city of Thessaloniki: the effects of wind direction, speed and persistence*. Int J Biometeorol, 49, 139-145.

[38] Díaz de la Guardia, C., Alba, F., Trigo, M. M., Galán, C., Ruiz, L. & Sabariego, S. (2003). *Aerobiological analysis of Olea europaea L. pollen in different localities of southern Spain*. Forecasting models. Grana, 42, 234-243.

[39] Waisel, Y., Mienis, Z., Kosman, E. & Geller-Bernstein, C. (2004). *The partial contribution of specific airborne pollen to pollen induced allergy*. Aerobiologia, 20, 197-198.

[40] Feo Brito, F., Galindo Bonilla, P. A., García Rodríguez, R., Gómez Torrijos, E., Fernández Martínez, F., Fernández-Pacheco, R. & Delicado Gallego, A. (1998). *Pólenes alergénicos en Ciudad Real: Aerobiología e incidencia clínica.* Rev Esp Alergol Inmunol Clin, 13, 79-85.