



Fraxinus pollen and allergen concentrations in Ourense (South-western Europe)



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ABSTRACT

In temperate zones of North-Central Europe the sensitization to ash pollen is a recognized problem, also extended to the Northern areas of the Mediterranean basin. Some observations in Switzerland suggest that ash pollen season could be as important as birch pollen period. The allergenic significance of this pollen has been poorly studied in Southern Europe as the amounts of ash pollen are low. Due to the high degree of family relationship with the olive pollen major allergen (backed by a sequence identity of 88%), the *Fraxinus* pollen could be a significant cause of early respiratory allergy in sensitized people to olive pollen as consequence of cross-reactivity processes. Ash tree flowers in the Northwestern Spain during the winter months. The atmospheric presence of Ole e 1-like proteins (which could be related with the Fra a 1 presence) can be accurately detected using Ole e 1 antibodies. The correlation analysis showed high Spearman correlation coefficients between pollen content and rainfall ($R^2 = -0.333$, $p < 0.01$) or allergen concentration and maximum temperature ($R^2 = -0.271$, $p < 0.01$). In addition CCA analysis showed not significant differences ($p < 0.05$) between the component 1 and 2 variables. PCFA analysis plots showed that the allergen concentrations are related to the presence of the *Fraxinus* pollen in the air, facilitating the wind speed its submicronic allergen proteins dispersion. In order to forecast the *Fraxinus* allergy risk periods, two regression equations were developed with Adjusted R^2 values around 0.48–0.49. The *t*-test for dependent samples shows no significant differences between the observed data and the estimated by the equations. The combination of the airborne pollen content and the allergen quantification must be assessed in the epidemiologic study of allergic respiratory diseases.

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1. Introduction

Fraxinus is a deciduous wind-pollinated tree of the Oleaceae family widely extended in temperate zones of the North and Central Europe. In general, ash trees are one of the most frequent natural species of riversides forests (Costa et al., 1998) nonetheless, they are also commonly used as ornamental plants in parks and gardens of urban areas (Candau et al., 1994). In Ourense (North-western Spain), the presence of three ash species is recorded. *Fraxinus angustifolia* Vahl is part of forest or moisture areas and the three rivers crossing the city favours its predominance. The abundance of *F. excelsior* L. and *F. pennsylvanica* L. is lower as they are used as ornamentals plants in parks and gardens. *F. angustifolia* blooms from late December to February while the pollen period of *F. excelsior* and *F. pennsylvanica* takes place from late February to the end of March (Guerra et al., 1995; Jato et al., 2004).

The pollen of *Fraxinus* represents about the 2% of the annual total airborne pollen in Ourense. In the Northern Europe areas, the sensitization to ash pollen denote a recognized problem (Heinzerling et al., 2009; Smith et al., 2014). Some studies conducted in Switzerland suggest that ash pollen season could be as important as birch pollen period in some regions (Schmid-Grendelmeier et al., 1994). An 18–34% of allergies sensitization rates can be attributed to the ash tree in France and South Austria (Poncet et al., 2010; Hemmer et al., 2000). Its allergenic significance has been poorly studied in the Southern European areas since the ash pollen amounts in the atmosphere are low (Barderas et al., 2005). Nonetheless, due to the high degree of family relationship with the olive pollen major allergen (backed by a sequence identity of 88%) ash pollen becomes a significant cause of early respiratory allergy diseases in the Southern Europe as consequence of cross-reactivity processes in olive pollen sensitized people (Barderas et al., 2005). Some studies have demonstrated the presence of cross-reactivity between different species of the Oleaceae family (Kerneman et al., 1992; Obispo et al., 1993; Baldo et al., 1992; Vara et al., 2016).

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Recent findings revealed the presence of Ole e 1 homologs in pollen extracts of ash and privet at concentrations in the range detected in olive pollen extracts (Lombardero et al., 2002; González et al., 2000). In the Northern areas of the Mediterranean basin, the diagnosis of ash pollen allergy is made difficult due to the non-inclusion in current diagnostic assays and some cross-reactivity with minor allergens from Betulaceae (Hrabina et al., 2007).

Fraxinus pollen contains five principal allergens: Fra e 1, Fra e 2, Fra e 3 and Fra e 9 (Barderas et al., 2005; Palomares et al., 2005). Fra e 1 with a PM of 18–21 kDa is the major allergen of ash pollen-sensitized patients. Belongs to the olive tree Ole e 1-like protein family and shows a high degree of homology with proteins from other Oleaceae members (Barderas et al., 2006). Approximately about 70–80% of ash pollen-sensitized individuals has Fra e 1-specific IgE antibodies (Barderas et al., 2005). Fra e 2 is a profiling of 13–16 kDa to which 50% of the ash-allergic patients have shown to be sensitized (Niederberger et al., 2002). Fra e 3 is a calcium-binding protein of 10 kDa (Niederberger et al., 2002). Fra e 9 is a 1,3-beta-glucanase, enzyme type widely distributed in higher plants and considered to be an important allergen (Palomares et al., 2005).

Previous studies conducted by means a combination of TEM and immunocytochemical techniques with anthers of *Fraxinus angustifolia* at different developmental stages and Ole e 1 antibodies (Rodríguez-Rajo et al., 2010) detected the presence of Ole e 1-like proteins in all microsporogenesis stages of the pollen grains. These identified proteins could be related with the Fra e 1 presence. The aim of the paper was to complement the study and to assess the presence of the *Fraxinus* pollen and its aeroallergens in the atmosphere as well as to evaluate its relationship with the main meteorological parameters. This information could help us to improve the prevention of clinical symptoms in pollinosis patients.

2. Materials and methods

The study was conducted in Ourense (42°0'N 7°5'W), located in Northwestern Spain at the bioclimatic Mediterranean region. The area has an annual mean temperature of 14 °C and 772 mm of annual total precipitation (Martínez-Cortizas and Pérez-Alberti, 1999). The *Fraxinus* pollen and its allergenic proteins in the atmosphere of this city were analysed during the 2009–2013 period.

Pollen sampling was carry out by using a Hirst type LANZONI VPPS 2000 volumetric sampler (Hirst, 1952) working continuously with a suction flow rate of 10 L/min in order to simulate the human breath. The sampler was located on the roof of the Science Faculty building of Ourense, approximately 15 m above the ground level. Pollen grains were captured using a Melinex tape coated with an adhesive solution. The samples were mounted in glass slides with glycerogelatine-basic fuchsin. The counting and identification of the pollen grains methods were performed using an optical microscope with a magnification of 40×, following the method of the Spanish Aerobiology Network (Galán et al., 2007). The pollen counts were expressed as grains per cubic meter of air. The atmospheric pollen season (APS) is considered as the period during which the 95% of the annual total accumulated sum of pollen is recorded. APS start when the accumulated sum of pollen reaches the 2.5% of the annual total and finish the day when the 97.5% of the annual total is reached. To calculate the risk of pollen allergy, the Spanish Aerobiology Network established the risk threshold in four groups, depending of the taxa (Galán et al., 2007). *Fraxinus* belongs to the group 3 which include three categories: Low (1–30 grains/m³), Moderate (31–50 grains/m³) and High (> 50 grains/m³).

For the aeroallergen quantification of the atmospheric aerosol, a Multi-Vial Cyclone Sampler (Burkard Manufacturing Co Ltd, Hertfordshire, UK) was used. The sampler was also located on the roof of the Science Faculty building of Ourense 2 m. next to the pollen trap. The sampler is a continuously wind-oriented device which uses a single reverse-flow miniature cyclone with an air flow rate of 16 L/min. The air samples were collected directly into a 1.5 mL Eppendorf vials during the *Fraxinus* pollen period, from December to April. Daily dry samples were stored at –20 °C. Airborne samples collected in 1.5 mL Eppendorf vials were analysed following the method described for Takahashi et al. (2001) with some modifications (Moreno-Grau et al., 2006). After centrifugation at 13,400 rpm for 3 minutes, dry samples were extracted with 120 µL of phosphate buffer (50 mM/L pH 7.0) that contained 150 mM/L NaCl, 125 mM/L ammonium bicarbonate, 3 mM/L EDTA and 0.005% Tween 20. The mixture was stirred continuously for 2 h at room temperature. The extract was separated for the particulate matter by centrifugation at 4000 rpm for 10 min and stored at –20 °C.

The Fra e 1 content in aerosol samples was quantified using specific 2-site ELISA and Ole e 1 antibodies, due to the high homology between *Olea* and *Fraxinus* proteins. Microtiter plates (Deltalab S.L., Barcelona, Spain) were coated with 100 µL of the mouse anti-Ole e 1 monoclonal antibody 5A3 L-121 at 0.55 mg/ml (Bial Industrial Farmacéutica, Spain) in phosphate-buffered saline (PBS) and incubated overnight at room temperature in a moist chamber. The wells were blocked (200 µL/well) with PBS-BSA-T (contained PBS with 1% bovine serum albumine and 0.05% Tween 20) and incubated for 1 h at 37 °C. Afterward, the plates were incubated for 1 h at 37 °C with 100 µL/well of purified Ole e 1, starting from a 2.5 µg/mL stock of natural Ole e 1, tenfold serial dilutions were performed in PBS-BSA-T, containing 50–0 ng/mL. At the same time we added 100 µL/well of the extracted airborne sample. After three washes with 200 µL/well PBS-T the plates were incubated for 1 h at 37 °C with 100 µL/well of the biotinylated rabbit anti-Ole e 1 polyclonal antibody L-091 at 0.72 µg/µL (Bial Industrial Farmacéutica, Spain). After three washes with 200 µL/well PBS-T the plates were incubated for 1 h at 37 °C with 100 µL/well of streptavidin-conjugated peroxidase (0.25 µg/mL) (Sigma S-5512) in PBS-BSA-T. Finally, plates were incubated with 200 µL/well of o-phenylenediamine (Sigma-Fast TM o-phenylenediamine dihydrochloride tablet set Sigma p-9187) for 30 min at room temperature in the dark. This step serves to determine the enzyme activity of the monoclonal antibody conjugate. The reaction was stopped by adding 50 µL/well 3 M H₂SO₄ and the absorbance was then measured at 492 nm. Ole e 1, used as reference material, was purified from *Olea europaea* pollen by standard chromatographic methods (Arilla et al., 2002). The standard curve was constructed from ten points using a four-parameter logistic curve fit. Results are expressed in nanograms per milliliter, referring to the protein content of the standard preparation. Later, the data were transformed to nanograms per cubic meter in order to be compared with the pollen data.

Weather data were supplied by the Agencia Estatal de Meteorología (AEMET). The meteorological station was located in the Science Faculty building of Ourense next to the Hirst type volumetric sampler and the Burkard Cyclone sampler. Temperature (°C), relative humidity (%), rainfall (mm), dew point (°C), and wind speed (km/h) were the parameters considered.

Statistical analysis was performed by means a non-parametric Spearman's correlation test in order to analyse the correlations between the meteorological factors and daily pollen or allergens concentrations. Multidimensional methods such as Canonical Correlation Analysis (CCA) and Principal Components Factor Analysis (PCFA), were performed with the aim to detect any structure in the relationships between variables, as well as to categorize the

variables according to their influence on the dependent variables' variation (Tipping and Bishop, 1999). The advantage of this non-parametric and multidimensional analysis is its hardness when the number of variables is high but there are a scarce number of observations (Sabatier et al., 1989). Considering the results obtained by the correlation, CCA and PCFA analysis, a linear regression equation was developed in order to construct a model to predict the *Fraxinus* pollen and aeroallergen concentrations in the atmosphere of Ourense. The package STATISTICA 5.1 was used for the statistical analysis.

3. Results

Ash trees flowers during the winter months in the North-western Spain area. The atmospheric pollen season (APS) is long with an average duration of 79 days. The APS lasted from January 7th to March 14th as average, although the start of the pollen season could be advanced to the month of December. During the year 2010 the APS was delayed from January 22th to April 13th, whereas in 2013 the earliest pollen season occurs from December 26th to April 23th (Table 1). The Seasonal Pollen Index-SPI (calculated as the daily mean concentrations sums during the APS) amounted to an average of 882 pollen grains and 0.517 nanograms of allergen during the study years. The maximum total annual pollen concentrations were registered during the year 2012 with 1523 pollen grains whereas the maximum allergenic load was registered in 2010 with 0.983 nanograms. The maximum pollen and allergen daily peaks were registered the 2010 January 24th with 174 pollen/m³ and 0.136 ng/m³ (Table 1). The daily mean pollen concentration threshold with high allergenic risks was reached as average during 3 days, rising to 5 during the year 2012. The characteristics of the pollen and aeroallergen curves, rainfall and average temperatures are shown in Fig. 1. In general allergen values fitted airborne pollen concentrations. Likewise, important atmospheric washes induced by the rainfall were observed as precipitations were followed by reduced levels of pollen and allergens (Fig. 1).

The correlation analysis between the *Fraxinus* pollen or the allergen concentrations and the main meteorological variables (temperature, humidity, dew point, wind speed and rainfall) taking into account the whole data set period showed the higher significant degree of association between the pollen and the rainfall or humidity, whereas for the allergens with the maximum, mean temperatures and rainfall (Table 2). The sign of the correlations was negative in both cases. Otherwise, there is a high

positive degree of association between the daily *Fraxinus* pollen counts and the allergen levels (Fig. 2). As consequence of the strong variation of the correlation analysis along the years of the study, a principal components analysis (PCFA) was performed in order to a better comprehension of the meteorological influence in the pollen and allergen airborne concentrations. PCFA is a classificatory method which considers the influence of all weather variables in conjunction. The purpose of the analysis is to obtain a small number of linear combinations of the 9 variables which account for most of the variability in the data. In our study 4 components have been extracted, since they had eigenvalues greater than or equal to 1.0 and they account for together around the 88.7% of the variability in the original data (Table 3). This fact suggests that four hypothetical (background) variables explained the majority of total variance with an 11.3% loss of information. Most of variables have significant positive loadings in the first two Principal Components (PCs). In general the four PCs were correlated as follows: Component 1 average/minimum temperatures and dew point, Component 2 maximum temperature and relative humidity, Component 3 pollen and allergen values Component 4 wind speed and rainfall. In order to better see the relationship between pollen/allergens and meteorological parameters a plot of PC1 vs PC3 was conducted (as *Fraxinus* pollen and allergens load significantly at PC3). The results of the PCFA show a high positive degree of association between the daily *Fraxinus* pollen counts and the allergen levels (Fig. 3). A Canonical Correlation Analysis (CCA) was performed with the aim that the canonical variables maximized the correlation between two groups of variables: the first group in which pollen and allergen variables were included and other group with the whole meteorological variables. The CCA analysis showed a highest correlation between the two set of variables with high statistically significant correlations as the p-values obtained are lower than 0.05 (Table 4).

In order to predict the pollen and allergen concentrations two linear regression models were performed (Table 5). The model to forecast the pollen content in the air, considered as estimators the allergen concentration and the rainfall, explains the 48.4% of the variability of the pollen concentrations. The model to predict the aeroallergen concentrations in the air, included as independent variables the pollen counts and the maximum temperature. The model explains the 49.4% of the aeroallergen concentrations variability. The accuracy of the equations was verified by the comparison between the observed and predicted data in the Fig. 4. The real values fitted the estimated data by the models. Moreover, the good forecast behaviour of the model is also statistically demonstrated by means a *t*-test of dependent samples. There are not

Table 1

Start date, end date and length of the atmospheric pollen season (APS). total *Fraxinus* pollen (pollen), pollen peak (pollen/m³), pollen peak date. Total allergen (ng), allergen peak (ng/m³), allergen peak date. Number of days with average daily concentrations upper than 31–50 pollen/m³ and > 50 pollen/m³.

		2009	2010	2011	2012	2013	Average
APS	Start	6-Jan	22-Jan	7-Jan	6-Jan	26-Dec	7-Jan
	End	30-Mar	13-Apr	22-Mar	31-Mar	6-Mar	14-Mar
	Length	83	82	75	86	71	79
Total pollen		851	796	616	1523	625	882
Pollen peak day		111	174	89	100	106	116
Date peak day		17-Mar	24-Jan	20-Jan	7-Feb	2-Jan	01-Feb
Total allergen		0.286	0.983	0.487	0.630	0.198	0.517
Allergen peak day		0.041	0.136	0.068	0.088	0.030	0.073
Date peak day		19-Mar	24-Jan	12-Jan	8-Feb	2-Jan	31-Jan
Days 31–50 pollen/m ³		1	3	3	10	1	4
Days > 50 pollen/m ³		3	3	2	5	3	3

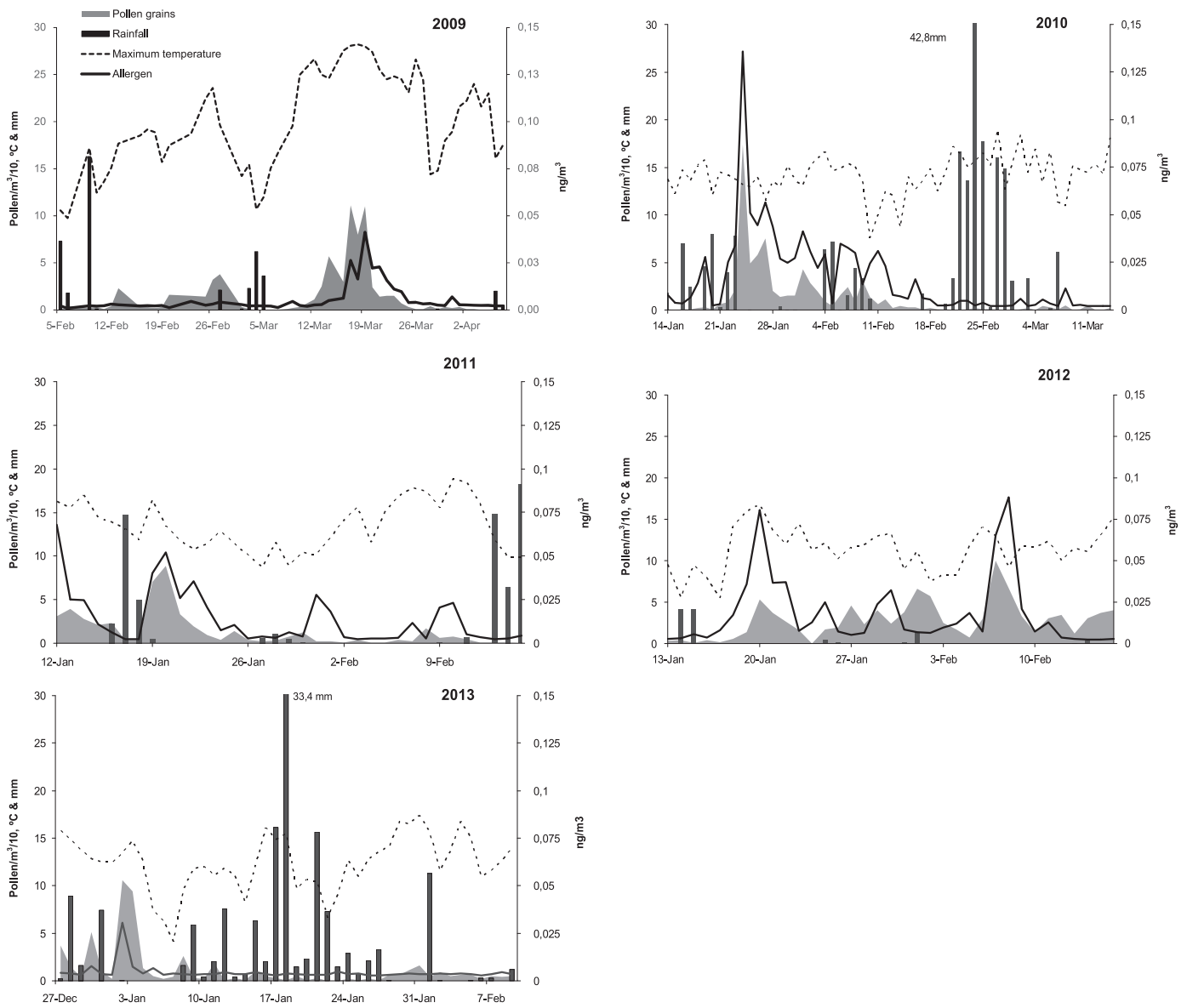


Fig. 1. Pollen grains/10 (grey area), Allergen concentration (black line), Maximum Temperature (black points) and Rainfall (bars) during the study period.

Table 2

Spearman correlations between *Fraxinus* pollen or allergen and the meteorological parameters during the APS.

	2009	2010	2011	2012	2013	2009–13
Pollen						
Mean temperature	0.417**	–0.258*	0.298*	–0.352**	0.317*	–0.065
Maximum temperature	0.656**	–0.264*	0.279	–0.245*	0.423*	0.016
Minimum temperature	–0.096	–0.170	0.215	–0.510**	0.224	–0.187**
Humidity	–0.547**	–0.178	–0.197	0.016	–0.433**	–0.195**
Dew point	–0.109	–0.182	0.257	–0.368**	0.146	–0.173**
Wind speed	–0.326*	–0.223	0.238	0.033	0.024	–0.181**
Rainfall	–0.428**	–0.242*	–0.237	–0.243*	–0.383**	–0.333**
Allergen						
Mean temperature	0.584**	–0.336**	0.154	–0.390**	–0.114	–0.194**
Maximum temperature	0.652**	–0.454**	0.142	–0.475**	0.027	–0.271**
Minimum temperature	–0.093	–0.173	0.138	–0.174	–0.083	–0.104
Humidity	–0.608**	–0.009	–0.327	0.364**	0.002	0.086
Dew point	–0.020	–0.207*	0.042	–0.031	–0.065	–0.066
Wind speed	0.054	–0.112	0.316	–0.029	–0.079	–0.059
Rainfall	–0.406**	–0.268*	–0.379*	–0.006	–0.296*	–0.152**

* $p < 0.05$.

** $p < 0.01$.

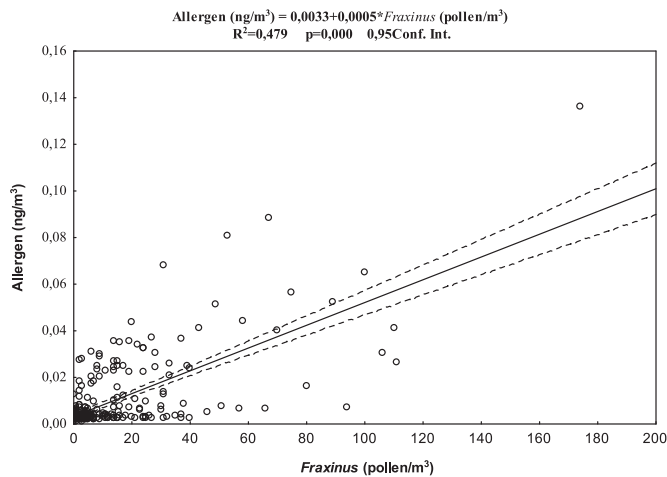


Fig. 2. *Fraxinus* pollen concentration (pollen/m³) versus allergen values (ng/m³).

Table 3
Principal components loadings (in bold $p < 0.05$).

Components	1	2	3	4
Eigenvalue	2.944	2.169	1.639	1.232
Percent of variance	32.711	24.103	18.208	13.689
Cumulative percentage	32.711	56.814	75.022	88.711
Allergen	−0.017	−0.084	0.923	0.029
Pollen	−0.069	0.056	0.910	−0.066
Average temperature	0.727	0.675	−0.046	0.061
Maximum temperature	0.292	0.900	−0.051	−0.086
Minimum temperature	0.930	0.119	−0.052	0.112
Relative humidity	0.348	−0.903	−0.027	−0.102
Dew point	0.970	−0.142	−0.035	−0.019
Wind speed	−0.069	0.157	0.053	0.920
Rainfall	0.406	−0.327	−0.180	0.632

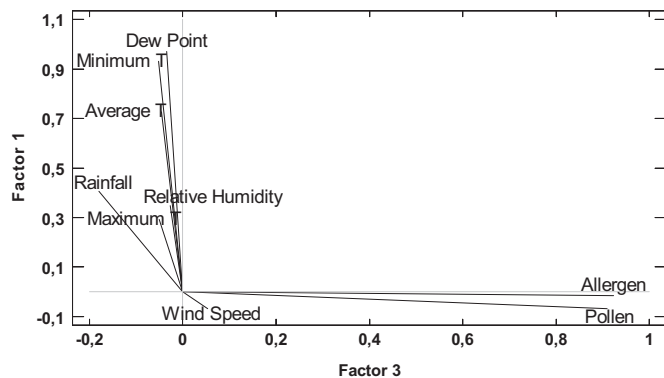


Fig. 3. Plot of principal components and factor analysis (PCFA). Factor loadings Factor 1 vs. Factor 3. Rotation varimax. Extraction Principal Components.

significant differences between predicted and observed pollen and allergen data (Pollen p -value 0.999, Allergen p -value 0.942).

4. Discussion

In the Northwestern Spain areas the ash pollen period takes place from late December to the months of March or April. The *Fraxinus* atmospheric pollen season is long due to the asynchronous pollen season of different ash species: *F. angustifolia* blooms from December to February and then takes place the pollen season of *F. excelsior* and *F. pennsylvanica* during the months of March and April (Guerra et al., 1995). Our study showed the higher amounts of ash pollen were registered from last December to early February

Table 4
Canonical correlation analysis ($p < 0.05$).

Set of variables	1	2
Eigenvalue	0.066	0.053
Canonical correlation	0.258	0.2303
p -value	0.001	0.016
Coefficients for canonical variables for Set 2		
Allergen	1.381	
Pollen	−0.872	
Coefficients for canonical variables for Set 1		
Average temperature	−1.036	
Maximum temperature	−0.794	
Minimum temperature	0.316	
Relative humidity	−0.867	
Dew point	1.248	
Wind speed	0.475	
Rainfall	−0.490	

as consequence of the *F. angustifolia* pollen emission (Jato et al., 2004). The maximum daily mean pollen an allergen peaks were delayed to the month of March due to the rainy days registered at the end of January and the beginning of February. The high humidity degree registered during winter in the Northwestern Spain and the existence of three rivers crossing the city, create the accurate conditions for the *F. angustifolia* growth. In contrast the high airborne pollen amounts are attributable in the Southern Spain areas to *F. excelsior*, as consequence of the low *F. pennsylvanica* representativeness and the gradual disappearance of the *F. angustifolia* wet distribution areas (Guerra et al., 1995). Furthermore, our study showed important atmospheric quantitative ash pollen values with regard to the Northern Europe areas (Barderas et al., 2005; Weryszko-Chmielewska et al., 2006; Smith et al., 2014).

Our finding shows that the atmospheric presence of Ole e 1-like proteins (which could be related with the Fra e 1 presence) can be also accurately detected using Ole e 1 antibodies. This fact recognized the major antigen of olive pollen (Ole e 1) as a main cause of cross-reactivity between Oleaceae pollens (Cariñanos et al., 2002; Niederberger et al., 2002; Obispo et al., 1993; Vara et al., 2016). The presence of Ole e 1-like proteins in all microsporogenesis stages of the *F. angustifolia* pollen grains ontogeny has been previously detected by means Ole e 1 antibodies (Rodríguez-Rajo et al., 2010). The localization of the Ole e 1-like protein in the tapetum cells as well as the important transfer of these proteins from the tapetum cells to microspores proves that this pollen protein is common in both gametophytic and sporophytic tissues (Rodríguez-Rajo et al., 2010).

Therefore, the sensitized people to the olive pollen grains could also suffer allergenic symptoms during the months of January–February as consequence of the *Fraxinus* pollen season. In general, the term ‘cross-reactivity’ should be used to describe clinical features showing the reactivity to a source without previous exposure (Ferreira et al., 2004). Some researches conducted in Northern Spain areas, where ash trees are common and olive trees are not represented, highlighted that olive pollen sensitized patients reported allergic symptoms during early spring coinciding with the ash pollen period (Gastaminza et al., 2005). In these areas, monosensitized patients to ash pollen allergens were also diagnosed (García et al., 2011). Therefore, Ole e 1 could be used as a biomarker allergen for the diagnosis of *Olea* and *Fraxinus* pollen allergy (Palomares et al., 2006).

Fraxinus flowering strongly depends on meteorological factors, mainly maximum temperature and rainfall (Jato et al., 2004). The ash pollen season should start at the end of December, when the temperature is elevate, but it may be delayed until the end of January if minimum temperatures below zero are recorded in

Table 5
Equations of the *Fraxinus* pollen (pollen grains/m³) and allergen (ng/m³) concentrations prediction. The Beta is the standardized regression coefficient, S.E. Beta is its standard error, B is the unstandardized regression coefficient and S.E. B is its standard error.

Pollen forecasting equation							
	R	R ²	Adjusted R ²	F	D.F	p-level	S.E.
<i>Fraxinus</i> pollen (pollen grains/m ³) = 4.245 + (966.072*Allergen) + (−0.395*Rainfall)	0.698	0.488	0.484	137.12	2.288	< 0.000	15.401
	Beta	S.E. Beta	B	S.E. B		t(288)	p-level
Intercepted			4.245	1.158		3.667	0.000
Allergen	0.681	0.043	966.072	60.387		15.998	0.000
Rainfall	−0.090	0.043	−0.395	0.186		−2.120	0.035
Allergen forecasting equation							
	R	R ²	Adjusted R ²	F	D.F	p-level	S.E.
Allergen (ng/m ³) = 0.0095 + (<i>Fraxinus</i> pollen*0.0005) + (Maximum Temperature*0.0004)	0.705	0.498	0.494	142.4	2.288	< 0.000	0.011
	Beta	S.E. Beta	B	S.E. B		t(288)	p-level
Intercepted			0.009	0.002		4.614	0.000
Pollen	0.694	0.042	0.0005	0.000		16.617	0.000
Max. temp.	−0.134	0.042	−0.0004	0.000		−3.200	0.002

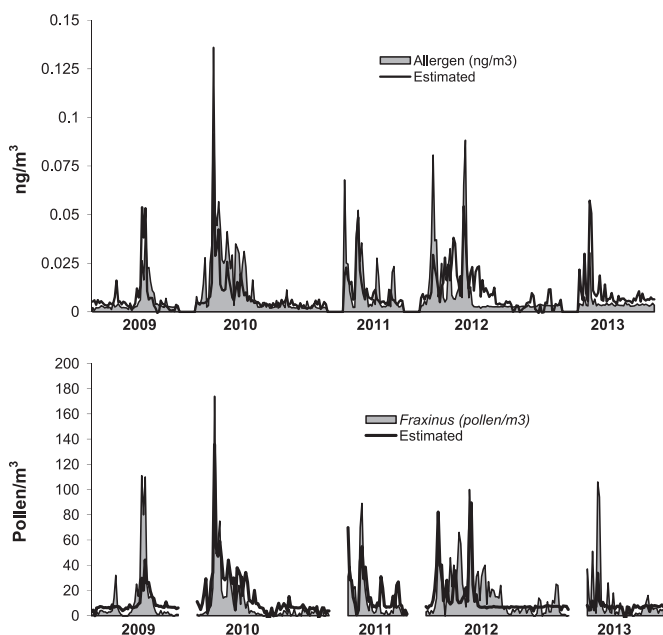


Fig. 4. *Fraxinus* pollen and allergen (in areas) and predicted values by the models (in lines).

December and/or early January (Jato et al., 2004). The correlation analysis between the *Fraxinus* pollen or the allergen concentrations and the main meteorological parameters varied along the years of the study as consequence of the strong meteorological inter annual differences observed during the winter period. Considering the complete data set, the correlation analysis showed high negative Spearman correlation coefficient between pollen content or allergen concentration and maximum temperature or rainfall. The unusual amount of total annual pollen grains observed in 2012 was consequence of the practically absence of rain registered during the APS, whereas the lower total amount of pollen observed in 2011 was produced by the high rainfall and lower temperatures registered during the *Fraxinus* APS. Similar results using Principal Component analysis have been reported by García-Mozo et al. (2008) for *Olea europea* and González-Parrado et al. (2009) for *Alnus*, noting that temperature and rainfall were the parameters that best explained the distribution of pollen. The PCAs plot indicated that allergen concentrations are strongly

related with the presence of the *Fraxinus* pollen in the air. Studies across Europe showed the birch pollen is the main source of its allergens in the atmosphere as they are only detected in the same fraction of the cascade samplers than pollen grains (Buters et al., 2012). Besides, the PCAs plot shows a relationship between pollen and allergens with the wind. Some studies conducted in the Southern part of the Iberian Peninsula highlights the role of the wind facilitating its dispersion (Silva Palacios et al., 2000). High-potency olive pollen was transported over 400 km to areas with low-potency pollen, where results in > 40% of the yearly exposure (Galan et al., 2013).

In order to forecast the *Fraxinus* allergy risk periods, two regression equations were developed. The adjusted R² values ranged around 0.48–0.49. The observed data fitted the graphical representation of the predicted values by the equations. The *t*-test for dependent samples shows no significant differences between estimated and observed data at the 95% level.

The Spanish Aerobiology Network established the *Fraxinus* pollen threshold of allergenic risks in three categories (Galán et al., 2007): Low (1–30 grains/m³), Moderate (31–50 grains/m³) and High (> 50 grains/m³). The use of the regression equations allowed us to calculate the corresponding aeroallergen allergy risk threshold concentrations: Low (0.000–0.024 ng/m³), Moderate (0.025–0.034 ng/m³), High (> 0.034 ng/m³). Both results help us to know the real number of risk days for the sensitized people as during the last years some authors (D'Amato, 2007) have observed the time of pollen exposure and the time when symptoms exist often do not coincide. Most recent studies have noted some discordances between the date of pollen and aeroallergen appearance in the atmosphere (D'Amato et al., 2008; Rodríguez-Rajo et al., 2011). In general, the aeroallergen peaks could be observed before the presence of pollen in the atmosphere (Cabrera et al., 2002). In our study the appearance of a first moderate aeroallergen risk event was detected five days before the first pollen peak in 2010. During this year 6 days with pollen allergy risk events has been found, nevertheless 18 days with aeroallergens allergy risk were detected (mainly twelve consecutive days from 22 of January to 2 of February). Studies conducted in Southern Spain also revealed yearly allergen exposure 3 times higher than pollen exposure; this effect would go unnoticed if only pollen was counted (Galan et al., 2013). In addition, some studies suggested that rainy episodes during the previous days of the atmospheric pollen peak concentrations could produce an increase of the aeroallergen

concentrations (D'Amato et al., 2007; Grote et al., 2003; Knox, 1993). The 2013 maximum daily aeroallergen peak was registered after previous rainfall episodes occurred two days before. Moreover, after short episodes of rain, pollen grains are deposited in different surfaces, and under these wet conditions, suffered a hydration that releases the allergenic proteins to the atmosphere (Moreno-Grau et al., 2006). When the 2011 *Fraxinus* APS was almost finishing under low pollen values, an aeroallergen peak with moderate risk of allergy was observed after four little rainfall episodes. Solomon et al. (1983) describe the violent expulsion of proteins resulting from the osmotic pressure and strong environmental evaporation during the following hours leads to rapid atmospheric dispersion of submicronic particles. These results show us the importance of the combination of pollen counts and aeroallergen quantification to investigate allergic respiratory diseases.

From a practical point of view, we consider that it is necessary to include a pollen extract of the Oleaceae family in the routine analytical prick-tests for the diagnosis of patients with sensitization to Oleaceae in areas where there is no olive pollen influence (Hrabina et al., 2007). There is evidence for high sensitization rates to ash in the Mediterranean-temperate borderlines (Guerra et al., 1995), possibly reflecting cross-reactivity in primary allergy to olive pollen (Hemmer et al., 2000). The combination of the pollen and allergen quantification must be assessed to reliably estimate the exposure of allergic people to allergens.

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References

- Arilla, M.C., Eraso, E., Ibarrola, I., Algorta, J., Martínez, A., Asturias, J.A., 2002. Monoclonal antibody-based method for measuring olive pollen major allergen Ole e 1. *Ann. Allerg. Asthma Immunol.* 89, 83–89.
- Baldo, B.A., Panzani, R.C., Bass, D., Zerboni, R., 1992. Olive (*Olea europaea*) and privet (*Ligustrum vulgare*) pollen allergens. Identification and cross-reactivity with grass pollen proteins. *Mol. Immunol.* 29, 1209–1218.
- Barderas, R., Purohit, A., Papanikolaou, I., Rodríguez, R., Pauli, G., Villalba, M., 2005. Cloning, expression, and clinical significance of the major allergen from ash pollen, Fra e 1. *J. Allergy Clin. Immunol.* 115, 351–357.
- Barderas, R., Purohit, A., Rodríguez, R., Pauli, G., Villalba, M., 2006. Isolation of the main allergen Fra e 1 from ASH (*Fraxinus excelsior*) pollen: comparison of the natural and recombinant forms. *Ann. Allerg. Asthma Immunol.* 96 (4), 557–563.
- Buters, J.T.M., Thibaudon, M., Smith, M., Kennedy, R., Rantio-Lehtimäki, A., Albertini, R., Reese, G., Weber, B., Galán, C., Brandao, R., Antunes, C.M., Jager, S., Berger, U., Celenk, S., Grewling, L., Jackowiak, B., Sauliene, I., Weichenmeier, I., Pusch, G., Sarioglu, H., Ueffing, M., Behrendt, H., Prank, M., Sofiev, M., Cecchi, L., 2012. Release of bet v 1 from birch pollen from 5 European countries. Results from the HIALINE study. *Atmos. Environ.* 55, 496–502.
- Cabrera, M., Martínez-Cócer, S., Fernández-Caldas, E., Carnés, J., Boluda, L., Tejada, J., et al., 2002. *Trisetum paniceum* (wild oats) pollen counts and aeroallergens in the ambient of Madrid. *Spain Int. Arch. Allergy Immunol.* 128, 123–129.
- Cariñanos, P., Alcázar, P., Galán, C., Domínguez, E., 2002. Privet pollen (*Ligustrum sp.*) as potential cause of pollinosis in the city of cordoba, south-west Spain. *Allergy* 57, 92–97.
- Candau, P., González Minero, F., Romero, F., 1994. Aero-palynology of *Fraxinus* (ASH) in an urban area of southwestern Spain. *Aerobiologia* 10, 47–51.
- Costa, M., Morla, C., Sainz, H., 1998. Los bosques Ibéricos. Planeta, Barcelona.
- D'Amato, G., 2007a. Allergenic pollen and pollen allergy in Europe. *Allergy* 62, 976–990.
- D'Amato, G., Liccardi, G., Frenguelli, G., 2007b. Thunderstorm-asthma and pollen allergy. *Allergy* 62 (1), 11–16.
- D'Amato, G., Cecchi, L., Liccardi, G., 2008. Thunder-storm-related asthma: not only grass pollen and spores. *J. Allergy Clin. Immunol.* 121, 537–538.
- Ferreira, F., Hawranek, T., Gruber, P., Wopfne, N., Mari, A., 2004. Allergic cross-reactivity: from gene to the clinic. *Allergy* 59, 243–267.
- Galán, C., Cariñanos, P., Alcázar, P., Domínguez, E., 2007. Spanish Aerobiology Network: Management and Quality Manual. University of Córdoba, Córdoba, pp. 1–61.
- Galán, C., Antunes, C., Brandao, R., Torres, C., García-Mozo, H., Caeiro, E., Ferro, R., Prank, M., Sofiev, M., Albertini, R., Berger, U., Cecchi, L., Celenk, S., Grewling, L., Jackowiak, B., Jäger, S., Kennedy, R., Rantio-Lehtimäki, A., Reese, G., Sauliene, I., Smith, M., Thibaudon, M., Weber, B., Weichenmeier, I., Pusch, G., Buters, J.T., 2013. Airborne olive pollen counts are not representative of exposure to the major olive allergen Ole e 1. *Allergy* 68, 809–812.
- García-Mozo, H., Pérez-Badía, R., Galán, C., 2008. Aerobiological and meteorological factors' influence on olive (*Olea europaea* L.) crop yield in Castilla-La Mancha (central Spain). *Aerobiologia* 24, 13–18.
- González-Parrado, Z., Valencia Barrera, R.M., Fuentes Rodríguez, C.R., Vega Maray, A.M., Pérez Romero, R., Fraile, R., Fernández González, D., 2009. Alternative statistical methods for interpreting airborne alder (*Alnus glutinosa* (L.) Gaertner) pollen concentrations. *Int. J. Biometeorol.* 53, 1–9.
- Gastaminza, G., Bartolome, B., Bernedo, N., Uriel, O., Audicana, M.T., Echenagusia, M.A., Fernández, E., Munoz, D., 2005. Oleaceae pollen allergy in a place where there's no olive trees. *Alergol. Immunol. Clin.* 20 (4), 131–138.
- García, B.E., Lizaso, M.T., Moreno, C., Rodríguez, R., Villalba, M.T., Ledesma, A., Tabar, A.I., 2011. Oleaceae-induced pollinosis in an area with exposure to olive and ash trees. *J. Invest. Allerg. Clin.* 21, 34–37.
- González, E.M., Villalba, M., Rodríguez, R., 2000. Allergenic cross-reactivity of olive pollen. *Allergy* 55, 658–663.
- Grote, M., Valenta, R., Reichelt, R., 2003. Abortive pollen germination: a mechanism of allergen release in birch, alder and hazel revealed by immunogold electron microscopy. *J. Allergy Clin. Immunol.* 111, 1017–1023.
- Guerra, F., Galán, C., Daza, J.C., Miguel, R., Moreno, C., González, J., Domínguez, E., 1995. Study of sensitivity to the pollen of *Fraxinus* spp. (Oleaceae) in Córdoba, Spain. *J. Invest. Allerg. Clin.* 5 (3), 166–170.
- Heinzerling, L.M., Burbach, G.J., Edenharter, G., Bachert, C., Bindslev-Jensen, C., Bonini, S., Bousquet, J., Bousquet-Rouanet, L., Bousquet, P.J., Bresciani, M., Bruno, A., Burney, P., Canonica, G.W., Darso, U., Demoly, P., Durham, S., Fokkens, W.J., Giavi, S., Gjomarkaj, M., Gramiccioni, C., Haahela, T., Kowalski, M.L., Magyar, P., Muraközi, G., Orosz, M., Papadopoulos, N.G., Röhnelt, C., Stingl, G., Todo-Bom, A., von Mutius, E., Wiesner, A., Wöhr, S., Zuberbier, T., 2009. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy* 64 (10), 1498–1506.
- Hemmer, W., Focke, M., Wantke, F., Gotz, M., Jarisch, R., Jager, S., Gotz, M., 2000. ASH (*Fraxinus excelsior*)-pollen allergy in central Europe: specific role of pollen panallergens and the major allergen of ash pollen, Fra e 1. *Allergy* 55 (10), 923–930.
- Hirst, J.M., 1952. An automatic volumetric spore trap. *Ann. Appl. Biol.* 39, 257–265.
- Hrabina, M., Purohit, A., Oster, J.P., Papanikolaou, I., Jain, K., Pascal, P., Sicard, H., Gouyon, B., Moingeon, P., Pauli, G., André, C., 2007. Standardization of an ash (*Fraxinus excelsior*) pollen allergen extract. *Int. Arch. Allergy Immunol.* 142, 11–18.
- Jato, V., Rodríguez-Rajo, F.J., Dacosta, N., Aira, M.J., 2004. Heat and chill requirements of *Fraxinus* flowering in Galicia (NW Spain). *Grana* 43, 217–223.
- Kerneman, S.M., McCullough, J., Green, J., Ownby, D.R., 1992. Evidence of cross-reactivity between olive, ash, privet, and Russian olive tree pollen allergens. *Ann. Allergy* 69, 493–496.
- Knox, R.B., 1993. Grass pollen, thunderstorms and asthma. *Rev. Clin. Exp. Allergy* 23 (5), 354–359.
- Lombardero, M., Obispo, T.M., Calabozo, B., Lezaun, A., Polo, F., Barber, D., 2002. Cross-reactivity between olive and other species. Role of Ole e 1-related proteins. *Allergy* 57, 29–34.
- Martínez-Cortizas, A., Pérez-Alberti, A., 1999. Atlas Climático DE Galicia. In: de Galicia, Xunta (ed.), Santiago Compostela, pp. 77–105.
- Moreno-Grau, S., Elvira-Rendueles, B., Moreno, J., García-Sánchez, A., Vergara, N., Asturias, J.A., et al., 2006. Correlation between *Olea europaea* and *Parietaria judaica* pollen counts and quantification of their major allergens OLE e 1 and par j 1-Par j 2. *Ann. Allerg. Asthma Immunol.* 96, 858–864.
- Niederberger, V., Purohit, A., Oster, J.P., Spitzauer, S., Valenta, R., Pauli, G., 2002. The allergen profile of ASH (*Fraxinus excelsior*) pollen: cross-reactivity with allergens from various plant species. *Clin. Exp. Allergy* 32, 933–941.
- Obispo, T.M., Melero, J.A., Carpizo, J.A., Carreira, J., Lombardero, M., 1993. The main allergen of *Olea europaea* (OLE e 1) is also present in other species of the Oleaceae family. *Clin. Exp. Allergy* 23, 311–316.
- Palomares, O., Villalba, M., Quiralte, J., Polo, F., Rodríguez, R., 2005. 1,3-beta-glucanases as candidates in latex-pollen-vegetable food cross-reactivity. *Clin. Exp. Allergy* 35 (3), 345–351.
- Palomares, O., Swoboda, I., Villalba, M., Balic, N., Spitzauer, S., Rodríguez, R., Valenta, R., 2006. The major allergen of olive pollen Ole e 1 is a diagnostic marker for sensitization to Oleaceae. *Int. Arch. Allergy Immunol.* 141 (2), 110–118.
- Poncet, P., Senechal, H., Clement, G., Purohit, A., Sutra, J.P., Desvaux, F.X., Wal, J.M., Pauli, G., Peltre, G., Gougeon, M.L., 2010. Evaluation of ash pollen sensitization pattern using proteomic approach with individual sera from allergic patients. *Allergy* 65, 571–580.
- Rodríguez-Rajo, F.J., Vega-Maray, A., Asturias, J.A., Jato, V., Seoane-Camba, J.A., Suárez-Cervera, M., 2010. The relationship between tapetum cells and microspores based on protein localization in *Fraxinus angustifolia* (Oleaceae) pollen grains. *Int. J. Plant Sci.* 171, 34–52.
- Rodríguez-Rajo, F.J., Jato, V., González-Parrado, Z., Elvira-Rendueles, B., Moreno-Grau, S., Vega-Maray, A., Fernández-González, D., Suárez, M., 2011. The combination of airborne pollen and allergen quantification to reliably assess the real pollinosis risk in different bioclimatic areas. *Aerobiologia* 27, 1–12.
- Sabatier, R., Lebreton, J.D., Chessel, D., 1989. Principal component analysis with instrumental variables as a tool for modelling composition data. In: Coppi, R., Bolasco, S. (Eds.), *Multiway Data Analysis*. Elsevier Science Publishers B.V., North-Holland, pp. 341–352.

- Silva Palacios, I., Tormo Molina, R., Muñoz Rodríguez, A.F., 2000. Influence of wind direction on pollen concentration in the atmosphere. *Int. J. Biometeorol.* 44 (3), 128–133.
- Schmid-Grendelmeier, O., Peeters, A.G., Wahl, R., Wüthrich, B., 1994. Zur Bedeutung der Eschenpollenallergie. *Allergologie* 11, 535–542.
- Smith, M., Jäger, S., Berger, U., Šikoparija, B., Hallsdóttir, M., Sauliene, I., Bergmann, K.-C., Pashley, C.H., de Weger, L., Majkowska-Wojciechowska, B., Rybniček, O., Thibaudon, M., Gehrig, R., Bonini, M., Yankova, R., Damialis, A., Vokou, D., Gutiérrez Bustillo, A.M., Hoffmann-Sommergruber, K., van Ree, R., 2014. Geographic and temporal variations in pollen exposure across Europe. *Allergy* 69 (7), 913–923.
- Solomon, W.R., Burge, H.A., Muilenberg, M.L., 1983. Allergen carriage by atmospheric aerosol, I: ragweed pollen determinants in smaller micronic fractions. *J. Allergy Clin. Immun.* 72, 443–447.
- Takahashi, Y., Ohashi, T., Nagoya, T., Sakaguchi, M., Yasu-eda, H., Nitta, H., 2001. Possibility of real-time measurement of an airborne *Cryptomeria japonica* pollen allergen based on the principle of surface plasmon resonance. *Aerobiologia* 17 (4), 313–318.
- Tipping, M.E., Bishop, C.M., 1999. Probabilistic principal component analysis. *J. R. Stat. Soc. B* 61, 611–622.
- Vara, A., Fernández-González, M., Aira, M.J., Rodríguez-Rajo, F.J., 2016. Oleaceae cross-reactions as potential pollinosis cause in urban areas. *Sci. Total Environ.* 542, 435–440.
- Weryszko-Chmielewska, E., Puc, M., Piotrowska, K., 2006. Effect of meteorological factors on *Betula*, *Fraxinus* and *Quercus* pollen concentrations in the atmosphere of Lublin and Szczecin. *Pol. Ann. Agr. Env. Med.* 13, 243–249.