

Are the profilins an important component in the atmosphere? Ole e 2-like panallergen

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Abstract The analysis of profilin Ole e 2 concentration and its relationship with pollen concentration was carried out in the atmosphere of León (located in north-western of Spain) during 3 years. Moreover, we have studied the influence of some atmospheric pollutants and climatic parameters using Spearman rank correlation and principal components analysis. As a result, the presence in the atmosphere of Ole e 2 is demonstrated. We can observe that in April and until mid-May, the peaks of Ole e 2 correspond to the high concentrations of pollen from various tree plants, such as *Quercus*, *Populus*, *Betula*, *Cupressaceae*. From this date until the middle of June, where the presence of this profilin begins to be scarce in the air, the concentration of Ole e 2 is linked to herbaceous plants: *Rumex*, *Plantago* and species of the family *Poaceae*. Statistical analysis showed that the factors of temperature get influenced the most in the presence of Ole e 2 in the atmosphere, followed by the relative

humidity and precipitation. In our study, there was no clear correlation with atmospheric pollutants; however, an increase in NO₂ and SO₂ causes a decrease in profilin in the atmosphere.

Keywords Ole e 2 profilin · Pollen · Atmospheric pollutants · Meteorological factors · PCA

1 Introduction

Respiratory allergic diseases, so common in Western countries, are increasing considerably in other parts of the world (D'Amato et al. 2007) and, despite advances in diagnosis and treatment of hay fever, clinical outcomes are not being met as expected. The true significance of the sensitivities obtained with routine diagnostic tests from pollen extracts is unknown. Therefore, various molecular diagnostic techniques have been applied to determine more precisely the cause of the allergic symptoms suffered by each patient (Valenta et al. 1991). They develop technical specifications from the deeper understanding of the biochemical structure and function of many plant allergens. On the other hand, it is checked that only the main pollen allergens do not play a key role in the origin of allergic symptoms (Valenta et al. 1992; Santos and Van Ree 2011). So it has been found that in certain geographic areas, minority allergens can be decisive in the appearance and/or amplification of

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symptoms in many patients, specifically the panallergens, i.e. allergenic proteins found in most related plants or phylogenetically and not only in the pollen grains where its amount is usually scarce, but also in other plant structures especially in fruits. One of those panallergens biochemically well studied is the profilin.

Profilin is one of the protein families most abundant in eukaryotic cells, both plant and animal (Rothkegel et al. 1996), as part of one group of proteins, most important ABPs (actin-binding proteins), and as described regulators, spatial and temporal changes in the organization and mechanical properties of actin cytoskeleton in response to cellular signals (Staiger 2000; Staiger and Blanchon 2006; Vidali and Hepler 2001). It has been shown that profilin plants, besides having a ubiquity, multifunctional and very soluble proteins character, are also allergens with high cross-reactivity between a wide range of species (Ledesma et al. 1998), and the polymorphic sequences showing several isoforms, as an adaptive advantage over various environmental conditions (Alché et al. 2007; Jimenez-Lopez et al. 2012). This molecular structure, which is well preserved in kingdom Plantae, is the base of natural cross-reactivity against pollen and plant food latex. Profilin pollen bind to IgE antibodies of allergic patients in varying proportions, which can reach more than 20%. These profilins increase the symptoms of type I allergy, and in some cases are major allergens (Barber et al. 2008; Rodríguez et al. 2002; Valenta et al. 1991). Recently it has been checked that profilins induce T cells activation and symptoms mediated by them (Jimenez-López et al. 2013).

In the province of León, as a result of the varied orography, climatology and edaphology in the study area, there are a significant number of vegetation series, and it is one of the provinces with the highest floristic richness of the entire Iberian Peninsula. In the city of Leon, flowering plants occur quickly due to weather conditions, and therefore, the pollen season is relatively short and intense, with high concentrations of pollen in the atmosphere between the months of April and June. Moreover, from the clinical point of view, the sensitization of patients to profilin in this area is relevant. A study of IgE response Mal d 4 showed that between 21 and 50% of patients with hay fever were sensitized to this panallergen.

We hypothesized that the presence of profilin in the atmosphere for periods of longer or shorter time, can

advance and/or amplify the allergic response-sensitive patients. In this paper, we analyse whether profilins presence is important in the atmosphere and analyse their release relate with respect to pollen concentration and with other environmental parameters. These results could show that profilins play an important role in the severity of respiratory allergies.

2 Materials and methods

2.1 Airborne pollen and aeroallergen data

Bioaerosol sampling was performed in Leon city, located in north-western Spain (42°34'N, 58°35'W), in the large province of Leon, with an area of 15,468 km² which belongs to the region of Castilla y León (together with another eight provinces).

The city is biogeographically situated in the Mediterranean region. León (830 m a.s.l.) has an annual mean temperature of 11 °C and 522 mm of annual precipitation, with a very irregular distribution throughout the year (with only 27,6 mm collected during the summer months). Pollen was sampled continuously during 2011, 2012 and 2013 using a Hirst-type volumetric sampler with a seven day recording trap (Burkard Manufacturing Co., Ltd. Hertfordshire, UK) placed on a terrace 15 m above ground level at León University Campus. The samples were prepared and analysed by the method of the Spanish Aerobiology Network (Galán et al. 2007). Pollen data were expressed as grains per cubic metre of air. Seasonal pollen integral (SPI_n) represents the total exposure to pollen from one season that was expressed as the integral sum of daily average pollen concentrations and carries the unit pollen*day/m³ (Galán et al. 2017). Pollen season has been defined as 3% to 97% of all pollen for that season.

Aeroallergens, like atmospheric dust, were sampled using a Burkard Multi-vial Cyclone sampler (Burkard Manufacturing Co Ltd. Hertfordshire, UK) that consists of a continuous volumetric sampler based on a single reverse-flow miniature cyclone (Lippmann and Chan 1979) with an airflow rate of 16.5 l/min. The efficiency is 99% for particle sizes up to 1.06 µm and 93% for particle sizes in the range 0.82–0.75 µm (Emberlin 1995). The atmospheric dust was collected directly into a 1.5-ml Eppendorf vial during the period 1 April to 16 August, when the largest number of

anemophilous species in this locality is flowering. Daily samples were stored at -20°C . The aeroallergen sampler was located at same terrace and within 5 m of a pollen sampler.

The results of the allergen concentration were expressed in picograms of allergen per cubic metre of air (pg/m^3). Seasonal allergen integral (SAIn) represents the total exposure to allergen from one season and carries the unit $\text{pg}\cdot\text{day}/\text{m}^3$.

2.2 Immunochemical aeroallergen quantification

The samples collected in Eppendorf tubes were extracted using the method as previously described (Fernández-González et al. 2010), in brief: after centrifugation ($18,000\times g$, 1 min), dry samples were extracted at room temperature for 2 h with 120 μL of phosphate buffer (50 mM pH 7.0) supplemented with 150 mM NaCl, 3 mM EDTA, 0.005% Tween 20 and 125 mM ammonium bicarbonate. The extract was separated by centrifugation at $2000\times g$ for 10 min and stored at -20°C . The profilin content in extracted samples was quantified using a double antibody sandwich ELISA method (Asturias et al. 1999). The ELISA plates (Greiner, Frickenhausen, Germany) were previously coated overnight with 0.25 $\mu\text{g}/\text{well}$ of monoclonal antibodies anti-profilin (3H8 and 5F2) (Bial Industrial Farmacéutica, Zamudio, Spain) at room temperature in a moist chamber and then blocked with 200 $\mu\text{L}/\text{well}$ of PBS-BSA-T (PBS with 1% bovine serum albumin with 0.05% Tween 20). Afterwards, plates were incubated with 100 μL per well of the extracted airborne samples, purified Ole e 2, starting from a stock of 1000 ng/ml , followed by 8 serial dilutions of 1/2 in PBS-BSA-T, as a reference curve and control plates were incubated with 100 $\mu\text{L}/\text{well}$ of biotinylated rabbit anti-Ole e 2 plates were incubated with 100 $\mu\text{L}/\text{well}$ of biotinylated rabbit anti-Ole e 2 polyclonal antibody (0.25 ng/ml) (Bial Industrial Farmacéutica, Spain) and then with 100 $\mu\text{L}/\text{well}$ of streptavidin-conjugated peroxidase (0.25 $\mu\text{g}/\text{ml}$ in PBS-BSA-T). All the incubations were performed at 37°C for 1 h, with 3 washes of 200 μL per well of PBS-B-T between successive steps. Finally, the enzyme activity was determined by adding 200 $\mu\text{L}/\text{well}$ of *o*-phenylenediamine (Sigma-FastTM tablet sets) and incubated for 30 min at room temperature in the dark. The standard curve was constructed from nine data points using a four parameter logistic curve

fit, and the concentration of crude extracts, assayed as dilution series, was interpolated from the linear portion of the standard curve and expressed in picograms per millilitre. Subsequently, the data were converted to picograms per cubic metre according to the volume sampled by the apparatus.

Ole e 2, used as reference material, was purified from *Olea europaea* var. *picual* pollen extract by poly-L-proline (PLP)-Sepharose 4B (Pharmacia, Uppsala, Sweden), by the method described by Arilla et al. (1997).

2.3 Environmental data

Weather data (daily maximum $^{\circ}\text{C}$, minimum $^{\circ}\text{C}$ and average temperature $^{\circ}\text{C}$, daily average humidity (RH) %, cumulative daily rainfall mm and solar radiation kJ/m^2) were provided by the close nearby station of the Spanish Meteorology Agency (AEMET). The pollutants data are obtained of the monitoring stations of urban city [NO_2 , O_3 , SO_2 , PM_{10} ($\mu\text{g}/\text{m}^3$)].

2.4 Statistical analysis

Data were compiled using Microsoft office Excel 07. We used Spearman's rank correlation coefficient for nonparametric data to analyse the correlations between environmental parameters and pollen/allergens concentration during the period April–August for each year (2011–2013). The level of significance was assessed between $p < 0.01$ and $p < 0.05$.

SPSS statistical software, version 21.0 (SPSS Inc, Chicago, IL), was used for all statistical analyses.

Principal components analysis (PCA) is often referred to and used as a method of factor analysis; it is not factor analysis at all. Nevertheless, some methodologists have argued that PCA is a reasonable substitute for analyses of common factors and might even be superior (Velicer and Jackson 1990). In this case, PCA was performed using the SPSS programme to reduce data set to a lower dimension and reveal some hidden and simplified structure that often underlie it (Worden and Farrar 2007). A VARIMAX rotation was used that is a change of coordinates used in principal components analysis (PCA) that maximizes the sum of the variances of the squared loadings. Thus, all the coefficients (squared correlation with factors) will be either large or near zero, with few intermediate values. The goal is to associate each variable to at most one factor.

3 Results

3.1 Pollen concentration

Given the previous database to this study (17 years: 1994–2010), in the city of León the mean of SPIn through that period was 13,770 pollen*day/m³, and the principal pollen period, following the established criteria, is relatively short; it extends from the first week of February until the end of August (Table 1). The number of pollen types present in the atmosphere of the city is 52 approximately, but *Betula*, *Cupressaceae*, *Oleaceae* (included *Fraxinus*, *Forsythia*, *Ligustrum* and *Olea*), *Pinus*, *Plantago*, *Poaceae*, *Populus*, *Quercus*, *Rumex* and *Urticaceae* contribute together 75% of the atmospheric concentration. Pollen emission patterns in León in 2011, 2012 and 2013 differ considerably between them. The seasonal pollen integral (pollen*day/m³) varied between 20,032 in 2011, 15,739 in 2012 and 16,316 in 2013; these values were higher than the average of the 17 previous years. During the period selected in this study, when pollinating most represented in the atmosphere species, i.e. from April 1 to August 16, the SPIn were 15,181 pollen*day/m³ in 2011, 10,663 pollen*day/m³ in 2012 and 13,757 pollen*day/m³ in 2013, corresponding to 76, 68 and 84%, respectively, of the total pollen (Table 1). The maximum pollen counts (pollen/m³), day of highest peak and the other aerobiological parameters of the different years of study are shown in Table 1 where we see that the start of the pollen period in 2012 was considerably delayed more than 15 days in relation to the other 2 years and 2013 includes the longest pollination period.

3.2 Airborne pollen allergen

The detection limit of the ELISA was 3.9 ng Ole e 2 ml⁻¹. During the period analysed, curves for pollen and profilin Ole e 2 concentration displayed similar behaviour, and allergen was found when no pollen was detected (Fig. 1). Cumulative Ole e 2 (pg*day/m³) is varied in the 3 years, 8572 in 2011, 3949 in 2012 and 2856 in 2013. The highest values of Ole e 2 were obtained on 24 May (482 pg/m³) in 2011 and 1 and 4 June (317,124 pg/m³) in 2012 and 2013, respectively. This occurs when the concentration of *Quercus* and grasses reaches very high values. In general, the main peak for allergen release did not coincide with the maximum values of pollen concentration (Table 1). In the 3 years studied, from the middle of June, the concentration of profilin decreased significantly.

3.3 Environmental data

In Table 2, we can see the environmental characteristics of the 3 years analysed, highlighting the amount of NO₂ produced in 2013 and the lower level of O₃ and PM10 in the same period. In 2011, rainfall was far superior to the other 2 years.

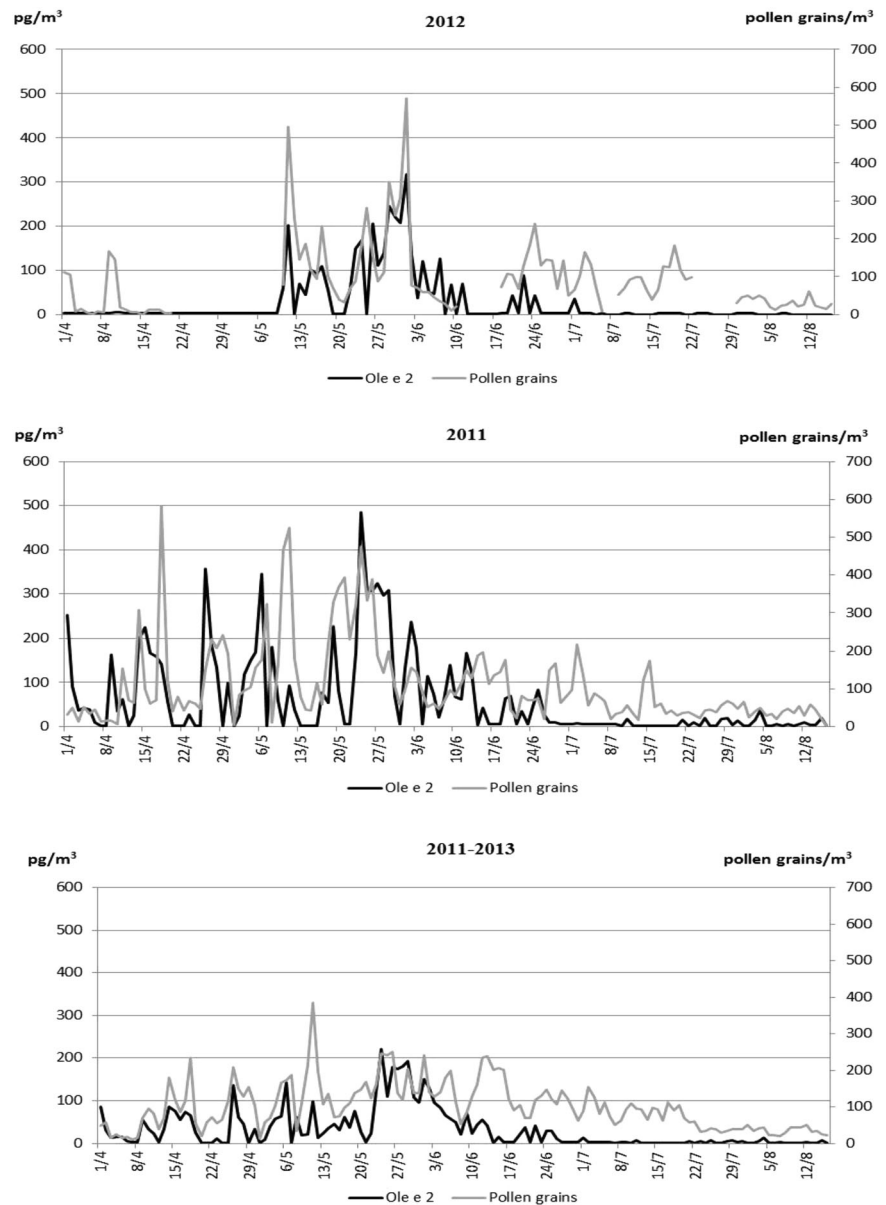
3.4 Statistical analysis

The nonparametric analysis Spearman's correlation was performed to analyse the association between the atmospheric concentration of Ole e 2 allergen and total pollen concentration (Table 3). Data were also used from other major pollen types in the atmosphere during the period of study as well as environmental parameters, meteorological: temperature, relative humidity, precipitation, solar radiation and the

Table 1 Pollen and allergen Ole e 2 data: SPIn (seasonal pollen integral, pollen*day/m³); SAIIn (seasonal allergen integral, allergen*day/m³)

Years	2011	2012	2013	2011/2013
PP (3–97%)	6 Feb–28 Aug	23 Feb–29 Aug	5 Feb–1 Sep	12 Feb–18 Aug
No. days	203	188	194	195
SPIn	15,181	10,663	13,757	13,770
Total pollen (%)	76%	68%	84%	80%
Data peak day	18 Apr	11 May	6 Jun	7 Jun
Peak day (pollen/m ³)	582	496	491	249
SAIIn	8572	3949	2856	
Data peak day	24 May	1 June	4 June	
Peak day (pg/m ³)	484	317	124	

Fig. 1 SPIn (pollen*day/ m^3) and Ole e 2 SAIIn ($\text{pg}^*\text{day}/\text{m}^3$) in the city of León (Spain) during 2011, 2012, 2013 and 2011–2013



pollutants NO_2 , O_3 , PM_{10} and SO_2 . In 2011, low significant correlations were obtained with the profilin and the most of pollen types, but in 2012 good associations with *Pinus* (0.619), *Quercus* (0.562) and *Rumex* (0.542) and in 2013 highest correlations were achieved with *Betula* (0.614) and *Quercus* (0.501). None of the studied years has appreciated good correlations with *Oleaceae* pollen type (Table 3).

Taking into account the meteorological and pollutants parameters for 2011 and 2013, a significant and low correlation was observed with the total pollen, O_3

and PM_{10} . In 2012, higher and different sign of correlations were appreciated with the PM_{10} particles, SO_2 , mean temperature, relative humidity and precipitation. In terms of environmental parameters, Ole e 2 was poorly correlated with the NO_2 , PM_{10} and minimum temperature during 2011; the value was very low and negative on this last case. In 2012, there was no significant correlation with almost any parameter, except for PM_{10} concentration, and in 2013, with O_3 and relative humidity but always with very low

Table 2 Data of average environmental characteristics during the period under study: nitrogen dioxide (NO₂) ozone (O₃), PM10, sulphur dioxide (SO₂), maximum temperature (*T* max), minimum temperature (*T* min), mean temperature (*T* mean), radiation (R), relative humidity (RH) and rainfall (P)

April–August	2011	2012	2013
NO ₂ (µg/m ³)	10	11	20
O ₃ (µg/m ³)	66	72	61
PM10 (µg/m ³)	21	21	16
SO ₂ (µg/m ³)	2	4	4.5
<i>T</i> max (°C)	23	21.7	21.5
<i>T</i> min (°C)	9.2	8.2	7.8
<i>T</i> mean (°C)	16	15	14
Radiation (kJ/m ²)	2503	–	2344
RH (%)	62	60	59
Rainfall (mm)	196	129	107

values and, a bit higher but the negative character, with the mean temperature (Table 4).

We have also carried out an analysis of main components, to better classify the influence of different variables on the allergen Ole 2. In Fig. 2, the results are shown. In the case of environmental factors, there have been extracted four main components explaining 82.2% of the variance. The four PCAs correlated were as follows: temperatures Component 1, Component 2 relative humidity, rainfall, and the radiation negatively, Component 3, SO₂ and NO₂ and Component 4, O₃ and PM10.

In relation to the various pollen types, three are the main components extracted that explain 62% of the variance. To perform the PCA, examining the previous statistical results, *Fraxinus*, characterized by early flowering, was separated from the remaining *Oleaceae*, to evaluate possible differences.

For the 3 PCAs (Fig. 3) high correlations were Component 1: *Quercus*, *Rumex*, *Pinus*, *Plantago* and *Oleaceae* (ex. *Fraxinus*), Component 2: *Fraxinus*, *Betula*, *Populus* and *Cupressaceae* and Component 3: *Poaceae* and *Urticaceae*.

4 Discussion

A large number of panallergens and specifically profilins have been generally described as responsible

Table 3 Spearman's correlation coefficients between allergen Ole e 2 and several pollen types during the period of study

Ole e 2	2011	2012	2013	2011–2013
<i>Betula</i>	.203*	.105	.614**	.172**
<i>Cupressaceae</i>	.197*	.193	.410**	.181**
<i>Fraxinus</i>	.060	– .080	.200*	.007
<i>Oleaceae</i>	.327**	.205*	– .086	.161**
<i>Pinus</i>	.409**	.619**	.400**	.335**
<i>Plantago</i>	.078	.496**	.124	.268**
<i>Poaceae</i>	.252**	.398**	.096	.196**
<i>Populus</i>	.106	– .184*	.280**	– .002
<i>Quercus</i>	.390**	.562**	.501**	.448**
<i>Rumex</i>	.405**	.542**	.201*	.345**
<i>Urticaceae</i>	– .171*	.007	– .251*	– .096
Total pollen	.463**	.451**	.409**	.417**

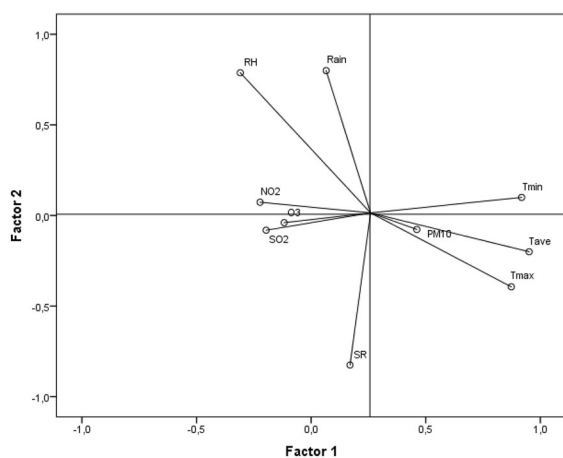
Significance level (**) 0.01 and (*) 0.05

for food allergic reactions and possible cross-reactions with respiratory allergies, all through clinical trials (Ferreira et al. 2004). In our study, the presence in the atmosphere of Ole e 2 is demonstrated. Its concentration, although variable in the 3 years analysed, is much lower than that of other airborne allergens, such as Par j 1–2 (Moreno-Grau et al. 2006), Pla a 1 (Fernández-González et al. 2010), Lol p 1 (De Linares et al. 2010; Fernández-González et al. 2011), Bet v 1 (Buters et al. 2012), Ole e 1 (Galán et al. 2013; Vara et al. 2016), Pla l 1 (González-Parrado et al. 2014), Phl p 5 (Buters et al. 2015; Plaza et al. 2016). In in vitro studies (Morales et al. 2008; Aloisi et al. 2018) have demonstrated that the Ole e 2 profilin is discharged into the medium in large quantities, after the hydration of the olive pollen, through the effective aperture for germination and remains largely adhered around said aperture and to the exine. Therefore, the recognition of the allergen by the polyclonal antibody Ole e 2 would be carried out mainly when the pollen of the *Oleaceae* family is present in the atmosphere and to a lesser extent would correspond to the exudates agglutinated or adhered to other particles that are in the air. However, in the 3 years of study, concentrations of the Ole e 2 allergen have not always been linked exclusively to the rate pollination of the *Oleaceae* family, but to the successive pollination of various

Table 4 Spearman's correlation coefficients between allergen Ole e 2, total pollen and environmental parameters

Year	2011 Pollen/Ole e 2	2012 Pollen/Ole e 2	2013 Pollen/Ole e 2	2011–2013 Pollen/Ole e 2
NO ₂	.096/.256**	.051/.142	.080/– .025	.079/– .188**
O ₃	.248**/.142	– .175*/.013	– .178*/– .230**	– .061/.014
PM10	.359**/.377**	.655**/.381**	.348**/– .101	.443**/.284**
SO ₂	.039/.053	– .469**/– .326**	.164/– .064	– .144**/– .423**
T mean	– .071/– .179*	.479**/.109	.253**/– .492**	.225**/– .127**
Radiation	.087/– .080	–	.184/– .123	.136*/– .040
RH	.047/.108	– .467**/– .099	– .268**/.297**	– .235**/.117*
Rainfall	.105/.112	– .328**/– .099	– .161/.140	– .149**/.018

Significance level (**) 0.01
and (*) 0.05

**Fig. 2** Principal components analysis (PCA) of environment factors affecting airborne Ole e 2 concentration: Factors from F1 to F4 ($p < 0.05$)

plant species. In any case, the presence of *Oleaceae* pollen in the atmosphere of the city of León is always low. The genera *Forsythia* and *Ligustrum* produce low quantity of pollen capable of dispersing in the atmosphere, and the genus *Olea* is not present in the city or in the province. The low concentrations of *Olea* pollen correspond to a long-distance transport, and only *Fraxinus* pre-spring pollination is well represented in the city. This is the most probable reason of low correlation between the pollen of *Oleaceae* family and the allergen Ole e 2. In April and until mid-May, the peaks of Ole e 2 observed correspond to the high concentrations of pollen from various tree plants, *Quercus*, *Populus*, *Betula*, *Cupressaceae* and, to a lesser extent, *Fraxinus*. From this date until the middle of June, where the presence of this profilin begins to be scarce in the air, the concentration of Ole e 2 is linked to herbaceous plants, *Rumex*, *Plantago* and species of

the family *Poaceae*. The analysis of PCAs reinforces these data. This would demonstrate that the polyclonal antibody Ole e 2 is able to recognize the profilin of *Quercus*, *Populus*, *Betula*, *Cupressaceae*, *Poaceae*, *Rumex* and *Plantago* pollen and the cross-reactivity among the main profilins of these plants. In fact, Ole e 2 is a profilin that presents a huge polymorphism at the molecular level and this polymorphism might represent a mechanism to generate multiple profilin isoforms among species (Jimenez-Lopez et al. 2012), due to stress or environmental changes in many cases. Thus, multimeric forms of profilins might constitute an additional mechanism of increasing the number of epitopes and variability that could produce in other plants, not only in olive tree. Furthermore, the differential recognition of plant profilin multimeric forms by immune system would not be a consequence of a simple additive effect. Otherwise, multimeric forms of profilin may operate synergically to facilitate the access of IgEs to defined epitopes in this big macromolecular complex (Jímenez-López et al. 2013). Therefore, the presence of Ole e 2 in the atmosphere of the Mediterranean regions, during long periods of the year, may be another important factor responsible of an increasing IgE-mediated risk of multiple pollen sensitizations.

The rapid solubilization and release of the allergenic proteins into aqueous media upon pollen hydration and activation is a typical characteristic that has been demonstrated in laboratory at the cellular level for several allergens of birch and grasses (Grote et al. 1993; Vrtala et al. 1993), *Parietaria* (Vega Maray et al. 2004), *Platanus* (Suárez-Cervera et al. 2005) and olive tree (Alché et al. 2004). However, the environmental characteristics for the release of allergens in the atmosphere are variables and not well known, as it

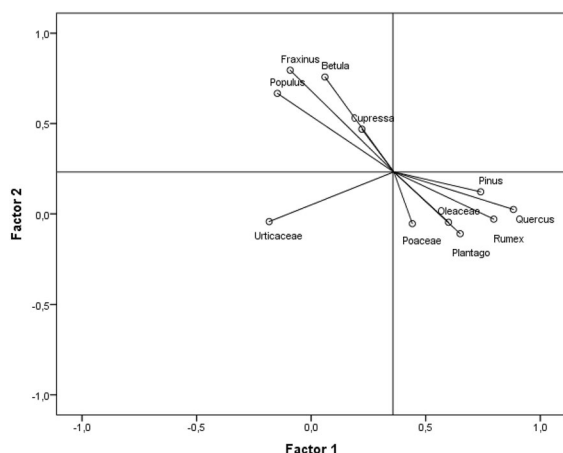


Fig. 3 Principal components analysis (PCA) of pollen types factors affecting airborne Ole e 2 concentration: Factors from F1 to F3 ($p < 0.05$)

is difficult to obtain water saturation level. On the other hand, the availability of water for the pollen once released is very variable since in the atmosphere, the wind speed and the high solar radiation stimulate the evaporation, so that pollen hydration is not carried out sequentially, but at certain times, so the allergen peaks do not always coincide with those of pollen (Aloisi et al. 2018). Some aerobiology studies mention specific levels of relative humidity and temperature in which the release of allergens occurs, as allergen levels in the atmosphere can be significantly increased after storms or rainy days (Solomon et al. 1983; Fernández-González et al. 2011). Other laboratory works show relative humidity percentages of approximately 40% and wind speed between 2 and 5 m/s to break the pollen grain by impact on some surface and release cytoplasmic granules that may increase the amount of allergens in urban atmosphere (Visez et al. 2015). In our study, we can confirm some of these data, since the highest SAI_n was reached in 2011, where the average temperature, rainfall and relative humidity were higher than the other 2 years as they favoured both the increased production of pollen that the release of profilin. Once reached the degree of adequate hydration, in 2013 due to the great drought that characterized the second half of May and the whole month of June, the presence of profilin in the air was practically null as of mid-June. The PCAs reinforce these data, since the environmental factors of temperature are those that get influenced the most in the presence of Ole e 2 in the atmosphere, followed by

those related to the presence of water, i.e. relative humidity and precipitation, as well as a high negative correlation with solar radiation.

The influence of certain atmospheric pollutants on the presence of pollen and/or allergens in the atmosphere is not clear and has been discussed in the literature in a very different way. An increase in SO₂ causes a decrease in the amount of soluble proteins contained in pollen (Cuinica et al. 2014), similarly to NO₂ (Sousa et al. 2012), but at the same time, this increase in NO₂ causes the nitration of certain allergenic proteins by altering the biochemical and immunological properties of the latter, which may be an increase in respiratory allergies (Ackaert et al. 2014). In our study, no clear correlations were observed about these factors, but the increase in NO₂ and SO₂ causes a general decrease (2011–2013) in profilin in the atmosphere, which corroborates the hypothesis of the decrease in proteins contained in the pollen under the influence of these pollutants. In general, the viability of pollen and germinative capacity, both in herbaceous and arboreal species, decreases with exposure to higher concentrations of O₃ that are within limits for human health (Chichiricco and Picozzi 2007; Sousa et al. 2012; Cuinica et al. 2014). Different concentrations of O₃ can modify the allergenic proteins (amino acids and peptides), such as oxidation (Sharma and Graham 2010). The influence of this gas on the concentration of Ole e 2 is not clear, although negative and lowest correlations between profilin and O₃ are observed in the year 2013, with lowest concentration of allergen.

Good correlations were observed with PM₁₀ particles with respect to both pollen and allergens. In relation to pollen and PM₁₀, both have the same dispersion model due to size and wind intensity. The case of Ole e 2 will probably aggregate and adhere to this kind of particles PM₁₀, as has been shown in some works (Laskin et al. 2016; Reinmuth-Selzle et al. 2017).

The great atmospheric instability that occurs in this region during the spring and that causes varied chemical reactions in the atmospheric aerosol can be the reason of the greater concentration of Ole e 2. This condition adds to the high metabolic activity of plants due to flowering, but also to their growth, since the profilin is involved in the regulation of the actin cytoskeleton dynamics (Kandasamy et al. 2002). At the end of the spring, the profilin in the air practically

disappears, although the concentration of some pollen types (e.g. *Poaceae*) continues being elevated.

The antigenic activity detected in additional parts in other plants and the presence of aeroallergens in structures different from pollen grains reinforce the need to quantify the bioaerosol allergens (D'Amato et al. 1991; Beggs 1998; Rantio-Lehtimäki et al. 1994; Buters et al. 2012, 2015; Galán et al. 2013; Raulf et al. 2014).

5 Conclusions

The results of this study demonstrate the important interaction between environmental factors and soluble proteins released by pollen. The increase in profilin in the air is directly linked to the increase of the relative humidity and PM10 particles. This is due to the gradual hydration of the pollen grain with the consequent metabolic activation and release of allergens.

The amount of Ole e 2 in the atmosphere depends on the concentration of total pollen, as there are numerous types of pollen that produce the similar profilins at the same time. These results also confirm the polymorphism of this protein, already observed biochemically.

The variability in the concentration of profilin during the studied years demonstrates the importance of the analysis of allergens as a complement to pollen counts and that all the pollen types present in the atmosphere should be considered when establishing levels of risk for patients with pollinosis. Numerous allergens and panallergens are proteins common to an important group of plant families due to their enzymatic and/or structural character.

In future studies, we should study in depth the protein content of each pollen type in order to analyse the time of hydration and emission of allergens in the pollen of various species. In this way, the interpretation of the discordances that appear in numerous occasions between the concentrations of pollen and those of allergens could be improved.

The presence of profilin throughout the study period serves to reinforce the hypothesis that these proteins are respiratory allergens of great clinical relevance.

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