



Atmospheric pollen allergen load and environmental patterns in central and southwestern Iberian Peninsula

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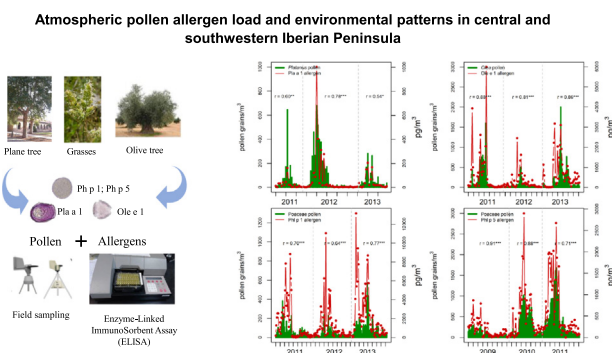
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HIGHLIGHTS

- The behaviour of atmospheric allergen and pollen concentrations is analysed
- ELISA method is applied to detect Pla a1, Ole e1, Ph p1 and Ph p5 allergens
- Mismatches in allergen-pollen ratio are affected by meteorological variables up to 6 days prior
- Same behaviour for Poaceae is found in the two areas studied in Spain and Portugal
- *Platanus* allergen-pollen ratio shows different patterns than *Olea* and *Poaceae*

GRAPHICAL ABSTRACT



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ABSTRACT

Over one quarter of the population in industrialised countries suffers from some type of allergy and inhaled aeroallergens from pollen are the primary cause of allergic ailments. The networks for monitoring biological air quality measure the airborne pollen concentrations that characterize periods of exposure to major airborne aeroallergens but there are certain discrepancies in relation to the allergen-pollen dynamic. In this paper we analyse the airborne allergens Ole e 1, Phl p 1, Phl p 5 and Pla a 1, and interpreted the adjustments and mismatches in their concentrations in relation to airborne pollen. The influence of main environmental patterns was considered. The study was conducted in two urban areas of the centre and southwest of the Iberian Peninsula (Toledo in Spain and Évora in Portugal). Monitoring for pollen followed the standard protocol using Hirst volumetric spore traps and allergenic particles were quantified by ELISA assay. The results indicate that the discrepancies in this relationship were affected by the weather conditions up to 6 days prior. Precipitation and humidity above normal values caused a higher concentration of the allergen Pla a 1. This effect occurred in reverse in the case of humidity for the allergens Ole e 1 and Phl p 1. Humidity and precipitation generated the same pattern in the allergen-pollen relationship in both Phl p 1 and Phl p 5. Our findings show consistent results that allow to interpret the rate of discrepancy between allergen and pollen, and it can be used to improve allergy risk prediction models generated from atmospheric pollen.

1. Introduction

Inhaled aeroallergens from pollen and spores are the primary cause of allergic ailments (Lake et al., 2017). According to the European Academy of

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Allergy and Clinical Immunology, over one quarter of the population in industrialised countries suffers from some type of allergy (European Academy of Allergy and Clinical Immunology, 2015). In addition, the incidence of respiratory diseases related to allergic reactions has increased in recent decades and continues to rise (Barnes, 2018; Cecchi et al., 2010; Reinmuth-Selzle et al., 2017), which has led to allergy being declared an important public health problem by the World Health Organization (Pawankar et al., 2013).

The networks for monitoring biological air quality measure the airborne pollen concentrations and characterize the periods in which the allergic population is exposed to the main aeroallergens (De Linares et al., 2019; Pérez-Badía et al., 2010). Allergy risk is evaluated indirectly by pollen airborne concentrations (Pfaar et al., 2019), primary due to historical reasons, when immunological detection techniques were not available. Nowadays, the quantification of airborne molecular allergens is not generally applied because of it implies a very laborious and costly analysis, but also, because there are allergenic pollen taxa for which molecular allergens have not yet been identified or fully characterised (Asam et al., 2015). In most cases, the relationships between allergic symptoms and airborne pollen concentrations have been far more documented than more “direct” indicators like exposure to molecular allergens (Bastl et al., 2015; Gökkaya et al., 2020). As a result, stations for monitoring pollen have become widespread throughout the world since the end of the 20th century (Buters et al., 2018) and automatic pollen control networks are beginning to be considered (Tummon et al., 2021).

In general, the presence of pollen and the allergen load in the air follow a similar dynamic (Buters et al., 2012; Vara et al., 2016). However, depending on the pollen type, certain discrepancies can be observed between pollen and allergen concentrations. These discrepancies have been observed in previous studies and can be attributed to meteorological factors, transport of free allergens after their release from the pollen grain or cross-reactivity with homologous allergens (Buters et al., 2015; Feo Brito et al., 2010; Plaza et al., 2016b; Ščevková et al., 2021). Nevertheless, the papers published to date show important gaps in the understanding of the factors that influence the relationship between pollen and allergen concentrations (Tegart et al., 2021).

The presence of large quantities of pollen in the air considerably increases the probability of finding high levels of allergenic proteins or allergens; however, aeroallergens can also travel freely or associated with other particles once they have been released from the pollen grain (Visez et al., 2015). Free aeroallergens are present in the atmosphere with a particle size in the fine fraction PM_{2.5} range and may bind to other fine particles such as the residual particles from diesel fuel combustion, other contaminants and even submicronic biological particles (Sénéchal et al., 2015; Visez et al., 2019). Pollen types with great importance as allergens come from the ornamental flora in cities, which, due to the abundance and proximity of the emission sources, are among the most common in the pollen spectrum in urban environments (Cariñanos and Casares-Porcel, 2011; Lara et al., 2019; Maya-Manzano et al., 2017). The plane trees (*Platanus* spp.) are species whose cultivation has become widespread in cities in Western Europe and whose pollen causes allergies in the months of March and April (Pérez-Badía et al., 2010; Rojo et al., 2016b). Pla a 1 is a major allergen that is recognized by up to 92 % of monosensitized *Platanus* allergic patients and 83 % of polysensitized patients (Asturias et al., 2002). The release process for the Pla a 1 allergen is associated with the hydration conditions of the pollen grain (Suárez-Cervera et al., 2005).

The main pollen types that cause pollinosis in the Mediterranean region are from grasses (Poaceae pollen type) and olive tree (*Olea* pollen type). Grasses are the primary cause of allergy worldwide (García-Mozo, 2017), and olive tree is very important in the whole of the Mediterranean basin as a consequence of the extensive distribution of this agricultural crop (D'Amato et al., 2007). In the case of the allergen groups of the grasses (Poaceae family), there is a predominance in the sensitization prevalence of Groups 1 and 5 (Andersson and Lidholm, 2003; Smith et al., 1994). Both groups include major allergens, specifically, the allergens of *Phleum pratense*, Phl p 1 (group 1) and Phl p 5 (group 5). The analysis of specific IgE permits to distinguish patients who have sensitization to these allergens that trigger allergic reactions in over 90 % (Phl p 1) and 65–85 % (Phl p 5),

respectively of people sensitive to grasses (Esch, 2004). In the case of olive, Ole e 1 is the major allergen, with 80 % of individuals allergic to olive sensitised to this allergen (Asam et al., 2015). Ole e 1 is involved in the hydration and germination of the pollen (Alché et al., 1999).

The main aim of this work was to analyse, in the atmosphere of urban areas in the centre and southwest of the Iberian Peninsula, (1) the relationship between the concentrations of major allergens (Pla a 1, Ole e 1, Phl p 1 and Phl p 5) measured from the air solid fractions and airborne pollen of plane tree (*Platanus* spp.), olive (*Olea europaea*), and grasses (Poaceae family); and (2) to identify and interpret the cases of discrepancy between allergen and pollen measurements in relation to the environmental patterns that influence the different phases of the aerobiological process. These environmental patterns are evaluated in both sampling sites of the Mediterranean region as a robustness test of the findings in the case of grasses.

2. Materials and methods

2.1. Study area

This work was carried out in central and southwestern Iberian Peninsula, in two cities, Toledo (Spain) and Évora (Portugal). Toledo is at 529 m and Évora at 300 m above sea level, with important bioclimatic differences between these two cities. In terms of climate, Toledo (Spain) has a mean average temperature of 15.8 °C and a mean annual precipitation of 342 mm (AEMET, 2022). The bioclimate is classified as Mediterranean pluvisseasonal-oceanic, the thermotype is Mesomediterranean, and the ombrotype is dry with a pronounced summer water deficit (Rivas-Martínez et al., 2017). In the case of Évora (Portugal), the bioclimate is also Mediterranean pluvisseasonal-oceanic, although the mean annual temperature is 15.9 °C with an annual precipitation of 609 mm (IPMA, 2022). It is located between the Meso- and Thermomediterranean belts in wetter conditions.

2.2. Aerobiological monitoring

The bioaerosol samples were obtained using different systems of capture. The aerobiological station (allergen and pollen samplers) is placed in the University of Castilla-La Mancha in Toledo (39° 51' N; 4° 02' W). In the case of Évora, the aerobiological station (allergen and pollen samplers) is placed in the University of Évora (38° 34' N; 7° 54' W) (a location map can be consulted in Supplementary Materials, Fig. S1). A Hirst-type volumetric (10 L/min) impaction sampler (Lanzoni Manufacturing s.r.l., Bologna, Italia) was used to capture airborne pollen samples in Toledo (Spain) and Évora (Portugal) (Hirst, 1952). The analysis of the samples and the identification and quantification of the pollen grains was done following the minimum requirements proposed by the International Association for Aerobiology (Galán et al., 2014). The pollen concentrations in the air were quantified daily in units of pollen grains/m³.

In parallel, samples of particulate matter were obtained to analyse the aeroallergens by means of two types of different volumetric traps. In Toledo (Spain), the bioaerosol sampling was done with a low-volume (16.5 L/min) multi-vial cyclone air sampler (Burkard Manufacturing Co. Ltd. Hertfordshire, UK), while in Évora (Portugal), a ChemVol® (Butraco Inc., Son, Netherlands) high-volume cascade impactor (800 L/min) was used. The ChemVol® sampled two different fractions of particulate matter (PM), one bigger than 10 µm and the other between 2.5 and 10 µm, although both fractions were summed to obtain the total amount of PM. The cyclone sampled particulate matter >1.06 µm.

The resolution of the airborne samples was daily, considering the “day” as the time from and to the midnight. The sampling period ran from 2009 to 2011 in Évora for grass pollen and allergens (Phl p 5), and from 2011 to 2013 in Toledo for pollen and allergens from plane tree (Pla a 1), olive (Ole e 1) and grasses (Phl p 1). Although aeroallergens were sampled using different sampling systems and during different sampling periods in Toledo and Évora, direct comparisons were avoided and just the same statistical procedure was applied to both locations in an independent way.

2.3. Extraction and quantification of aeroallergens

The aeroallergens present in the samples obtained from the cascade impactor (ChemVol) were extracted in ammonium bicarbonate (0.1 mol/L, pH 8.1) with 0.1 % BSA in rotation at 100 rpm for four hours (Buters et al., 2015).

The particulate matter samples collected in the cyclone sampler were extracted following the method described by Takahashi et al. (2001) with the modifications proposed by Moreno-Grau et al. (2006).

The allergens concentrations were quantified by Enzyme-Linked ImmunoSorbent Assay (ELISA) using specific antibodies (Arilla et al., 2002, 2005; Duffort et al., 2008). Phl p 5 was quantified in Évora and Pla a 1, Ole e 1 and Phl p 1 were quantified in Toledo. The allergen concentrations in the air were quantified daily in units of pg/m^3 .

2.4. Meteorological data

The meteorological data for Évora were supplied by the Atmospheric Sciences Observatory at the University of Évora ($38^\circ 34' \text{N}$; $7^\circ 54' \text{W}$) located at an altitude of 300 m above sea level. In Évora the meteorological station is located side-by-side with the aerobiological devices. In Toledo, the meteorological station ($39^\circ 53' \text{N}$; $04^\circ 03' \text{W}$) is located at 2.24 km from the aerobiological station, at an altitude of 515 m above sea level, belonging to the Spanish Meteorological Agency (www.aemet.es). The meteorological variables analysed in daily resolution were maximum temperature ($^\circ\text{C}$), minimum temperature ($^\circ\text{C}$), daily accumulated precipitation (mm) and relative humidity (%). Wind speed (m/s) was assessed in an exploratory analysis to evaluate the distribution of winds for each category of allergen-pollen ratio as well as the other meteorological variables (see Supplementary Materials, Fig. S2). Mean temperature and relative humidity were used together to characterize the average pollen curves during the study period.

The time series of meteorological data was decomposed (deseasonalised) following a process based on the LOESS algorithm. This type of disaggregation allows the seasonality and trend to be extracted in order to isolate the random component of the data series (Cleveland et al., 1990) which provides information on the meteorological anomaly (+ / -) in the seasonal behaviour of the variable. This procedure also makes it possible to correlate data from two time series, ensuring the temporal independence of the daily data in the case of stationary and strongly seasonal variables such as temperature and relative humidity (Lara et al., 2019).

2.5. Statistical analysis

The relation between allergen and pollen concentrations were analysed using linear regression between both measurements for each pollen type. This relation has a significance of the general allergenic potency of pollen for the different pollen types; that is, the allergen concentration corresponding to the pollen concentration for each day (allergen-pollen ratio). Moreover, correlations between allergen and pollen concentrations were determined year-by-year using Spearman's correlation test due to the fact that pollen and allergen concentrations do not follow a normal distribution. In the same way, the residuals of the linear regression in the allergen-pollen ratio do not follow a normal distribution and Spearman's correlation test was used as it is less sensitive to deviant cases.

Assuming a linear relationship, the residuals of the regression analysis – that is, the data located outside the 99 % confidence interval of the linear regression – allow us to identify discrepant cases. These cases outside the confidence interval can occur in two directions: days in which the allergen concentration exceeds the corresponding pollen concentration (“High” cases), and days when the pollen concentration exceeds the corresponding allergen concentration according to the 99 % confidence interval (“Low” cases). The remaining statistical analyses are based on the interpretation

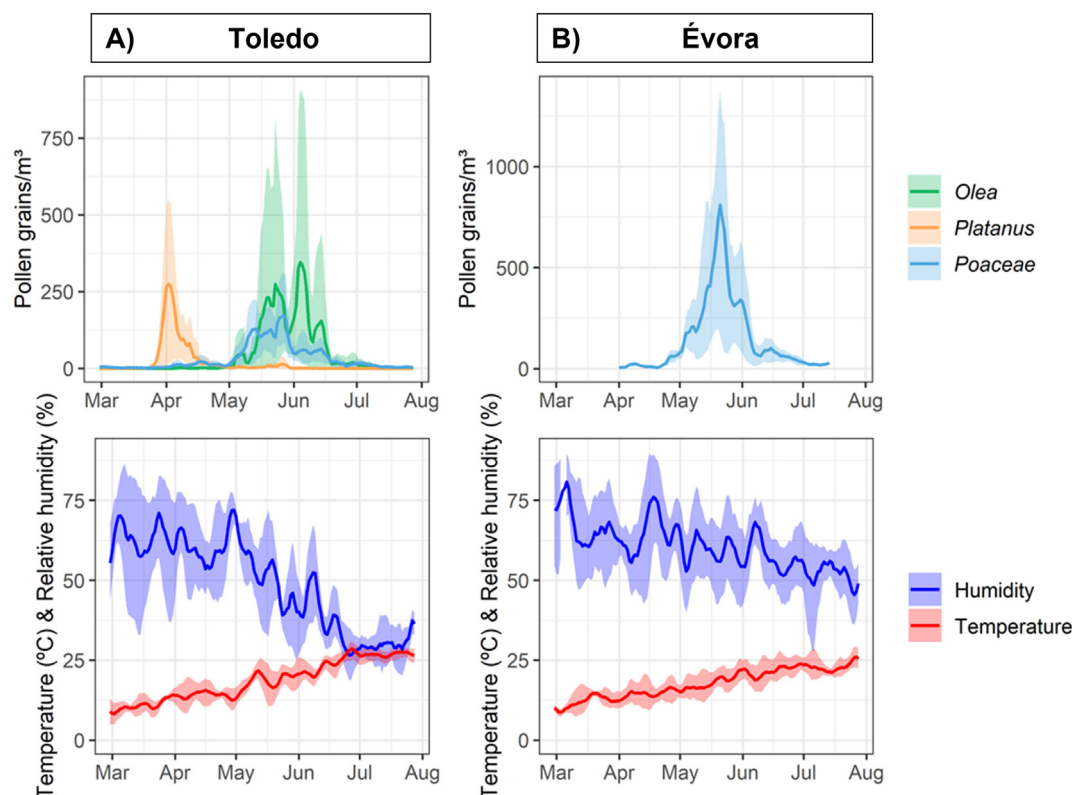


Fig. 1. Pollen concentration for *Platanus*, *Olea* and *Poaceae* pollen (pollen grains/ m^3), and meteorological variables of mean temperature ($^\circ\text{C}$) and relative humidity (%) in Toledo (A) and Évora (B). Average (line) and its amplitude (area) during the period 2011–2013 in Toledo, Spain, and the period 2009–2011 in Évora, Portugal.

of the residuals of the linear regression (discrepancies $\pm$ regarding 0 which correspond to the total correspondence between pollen and allergen).

In order to analyse the influence of meteorological variables on the allergen-pollen ratio, the residuals of the allergen-pollen in the regression analysis and the meteorological variables were studied using Spearman's correlation test. A 3-day moving average was previously used to smooth the effect in the very short-term with the objective of improving the interpretation. The meteorological values for the current day and 9 lags were used in the correlation test, i.e. the meteorological variables were studied with a delay of up to 9 previous days. All the statistical analyses were done with R Software (R Core Team, 2019).

3. Results

The pollen season for the three pollen types studied, *Platanus*, *Olea* and *Poaceae*, occurs between March and August, when practically the total pollen emitted into the air by the species producing this pollen is recorded (Fig. 1). However, *Platanus* has a much earlier pollen season than *Olea* and *Poaceae*, occurring between late March and April. During this period there is a gradual rise of temperatures in Toledo to around 13 °C and a mean relative humidity of around 50–75 %. The pollen season for *Olea* and *Poaceae* coincides closely in time, and the highest levels of these pollen types can be observed between May and June, although the maximum peak for *Poaceae* occurs before *Olea*. During this period in late spring and early summer, the rate of increase of the mean temperature accelerates and the relative mean humidity falls to approximately 25–50 % in Toledo, although not so drastically in Évora (Fig. 1).

The regression analysis of the pollen concentration of *Platanus*, *Olea* and *Poaceae* and the concentration of their respective allergens shows that the coefficients of determination (R^2) between the *Platanus* pollen and the allergen Pla a 1 is $R^2 = 0.57$ ($p < 0.01$) (Fig. 2). The coefficients of determination of the regressions between the pollen concentrations of *Olea* and the allergen Ole e 1 is $R^2 = 0.35$ ($p < 0.01$), and for the *Poaceae* pollen and the concentration of the allergen Phl p 1 and Phl p 5 it is $R^2 = 0.32$ ($p < 0.01$) and $R^2 = 0.59$ ($p < 0.01$), respectively. Although in general the degree of association between pollen and allergen concentration is high, and in all cases significant ($p < 0.01$) (Fig. 2), there are many daily cases that reveal far from the total correspondence (residuals of the linear regression). The allergen-pollen ratio “(pg/m³)/(pollen grain/m³)” is very different between sites with a value of 16.9 in Toledo and 1.3 in Évora (slopes of the linear regression) for *Poaceae*. It should be noted that a few cases exceed even the value of 10,000 pg/m³ for Phl p 1 in Toledo. The allergen-pollen ratios for *Olea* and *Platanus* are 1.8 and 0.7, respectively.

Figs. 3 and 4 show the annual correlation between *Platanus* pollen and the Pla a 1 allergen (Fig. 3A), *Olea* pollen and the Ole e 1 allergen (Fig. 3B), *Poaceae* pollen and the Phl p 1 allergen (Fig. 4A) and *Poaceae* pollen and the Phl p 5 allergen (Fig. 4B).

The annual correlation between the concentrations of *Platanus* pollen and the Pla a 1 allergen is high and always significant. Although the degree of correlation is less for the years 2011 and 2013 ($r = 0.60$, $p < 0.01$ and $r = 0.54$, $p < 0.05$ respectively), the maximum concentration peaks in both cases continue to coincide adequately (Fig. 3A). In the case of *Olea* pollen and its correlation with the Ole e 1 allergen is high in all years ($r > 0.80$, $p < 0.001$); that is, there is a close correlation between pollen and allergen levels, and the maximum annual peaks coincide for both measurements (Fig. 3B).

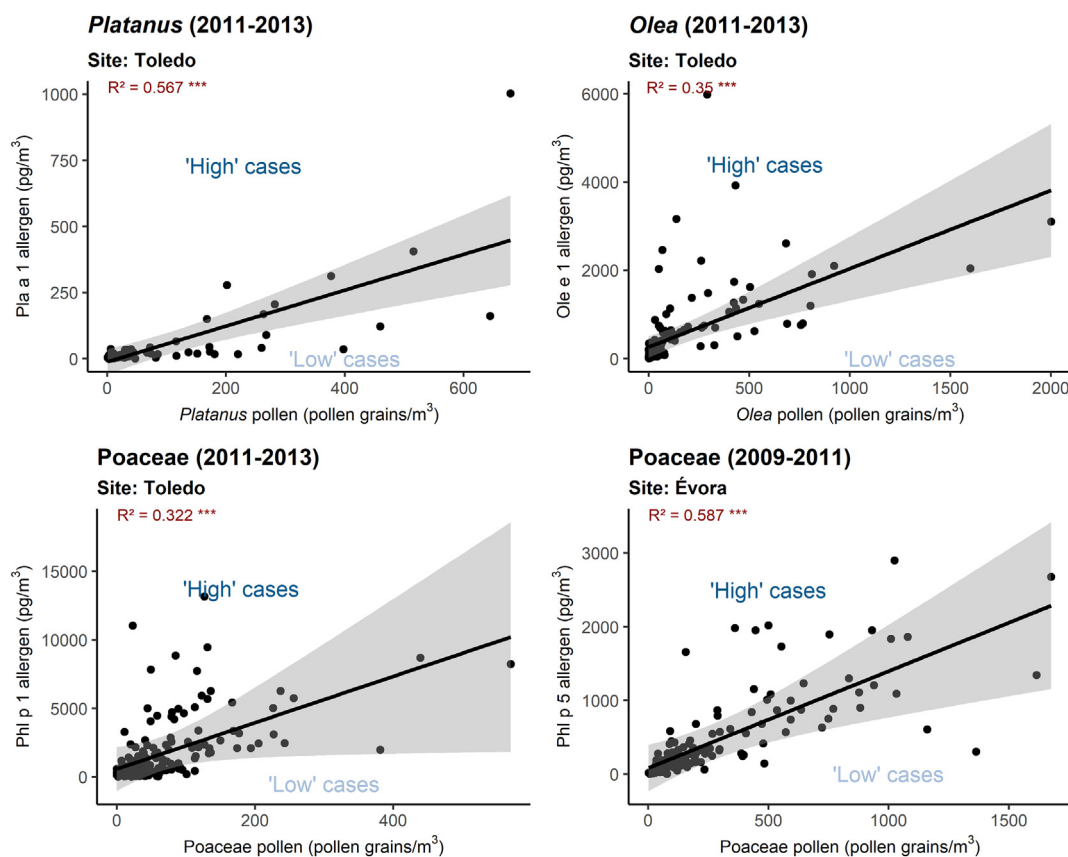


Fig. 2. Regression analysis for studied period comparing pollen concentration and its respective allergen concentration for (A) *Platanus*-Pla a 1 in Toledo, (B) *Olea*-Ole e 1 in Toledo, (C) *Poaceae*-Phl p 1 in Toledo, and (D) *Poaceae*-Phl p 5 in Évora. “High” cases correspond to positive residuals and “Low” cases correspond to negative residuals. R^2 Coefficient of determination.

The correlation between the concentrations of Poaceae pollen and the Phl p 1 allergen in Toledo and Phl p 5 in Évora is also high, although the degree of correlation is higher in Évora (0.71, 0.88 and 0.91, $p < 0.001$, respectively) (Fig. 4B), compared to Toledo (0.64, 0.70 and 0.77, $p < 0.001$, respectively) (Fig. 4A). In the case of Poaceae, there was a higher number of days with a greater concentration of Phl p 1 and Phl p 5 allergens in relation to the concentration of Poaceae pollen (Fig. 2, “High” cases according to the analysis of residuals in the linear regression).

Regarding the cases of pollen-allergen discrepancies in relation to the meteorological patterns, the correlations between the residuals of the allergen-pollen ratio and the meteorological variables show that there is a positive correlation for relative humidity and precipitation in the case of *Platanus* (Fig. 5). That is, the allergen-pollen ratio is greater when the humidity or precipitation is high, which means more discrepancies between pollen and allergen concentrations (“High” cases). In contrast, the temperature behaves in the opposite way, showing a negative relation with *Platanus*. It can also be seen that the intensity of the correlations gradually decreases until the effect disappears or even changes its sign as in the case of precipitation.

A comparison of the relation between *Olea* and Poaceae pollen-allergen ratio residual data from the regression analysis and the meteorological variables at different lags reveals a contrasting pattern as compared to *Platanus*. In the case of *Olea*, the significative correlation between relative humidity and precipitation and the allergen-pollen ratio is 1 to 6 and 3 to 7 days before, respectively, although in this case with a negative sign, unlike in the case of *Platanus* (Fig. 5).

It is also worth noting how the results of the correlations for relative humidity and rainfall were equivalent for the study of both allergens in Phl p 1 and Phl p 5 in Toledo (Spain) and Évora (Portugal) respectively (Fig. 5). In

both cities the same correlation pattern can be observed throughout the time delays, although this synchrony does not occur in the case of temperature.

When we look in detail at the effect of relative humidity on the allergen-pollen ratio for the pollen types and allergen studied (Fig. 6), we obtain the same results observed previously. Most of the days that show an allergen-pollen ratio within the confidence interval are related with a low range of anomalies in relative humidity (average 0 with regard to the humidity residual, Fig. 6). However, the days considered as “high” cases (the allergen concentration exceeds the pollen concentration) in *Olea* and Poaceae show a relation with negative anomalies for relative humidity on the previous day; that is, days with a significant decrease in humidity, unlike in “low” cases (Fig. 6).

In the case of *Platanus*, the relation is the opposite once again highlighting the differences between the pollen types of *Olea* and Poaceae with regard to *Platanus*. The “high” cases were observed on days with positive anomalies for relative humidity, i.e. greater relative humidity than the average seasonal value, although in no case with significant differences between groups (Fig. 6).

In the case of rainfall for olive and grasses, the days belonging to “low” cases (the pollen concentration exceeds the allergen concentration) in *Olea* and Poaceae show a relation with the number and intensity of rainfall events for the previous day. Also, the results of rainfall for two previous days show the same pattern for both in Toledo and Évora (Fig. 7).

4. Discussion

The concentrations of allergenic pollen recorded by the aerobiological monitoring networks have been widely used as indicators of the levels of

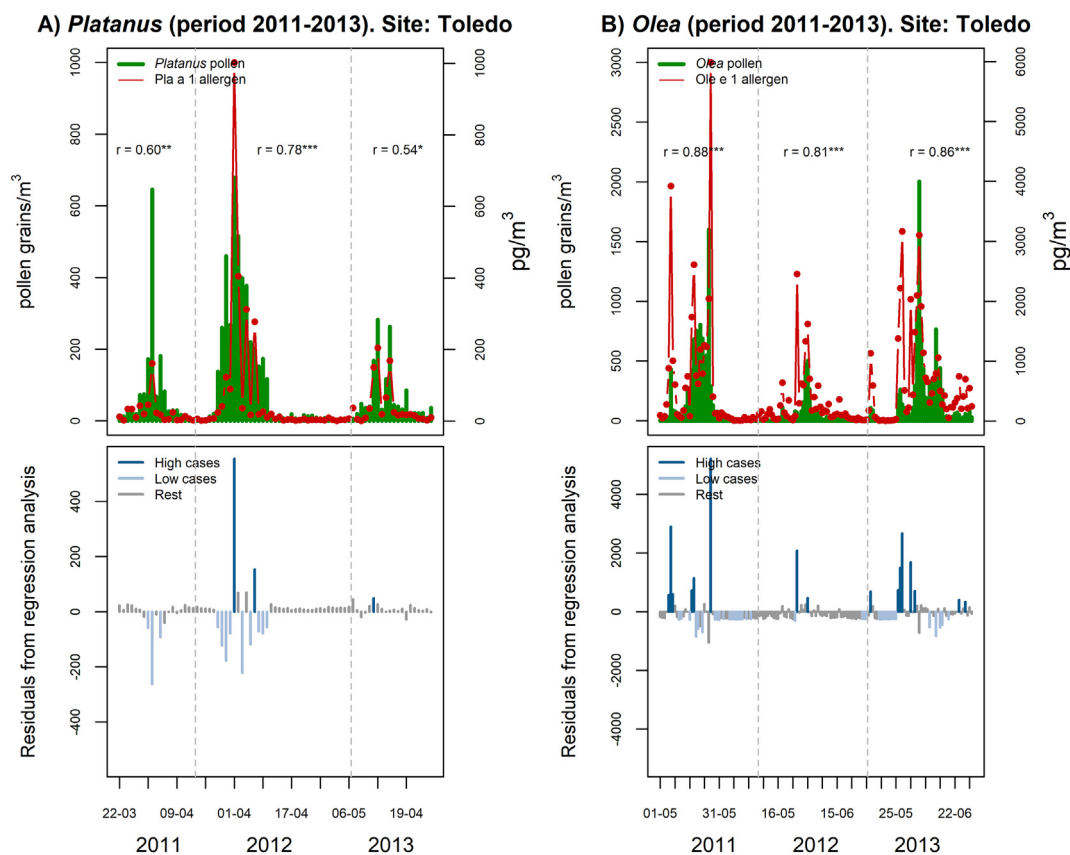


Fig. 3. Top: annual relationship between (A) *Platanus* pollen and Pla a 1 concentrations, and (B) *Olea* pollen and Ole e 1 concentrations in Toledo (Spain) during 2011–2013. r Spearman correlation test. Bottom: special cases outside the 99 % confidence interval. Value of the residuals is the difference between the observed and the obtained values of the regression analysis from Fig. 1. “High” cases correspond to positive residuals and “Low” cases correspond to negative residuals.

allergy risk around the world (Buters et al., 2018). However, certain doubts have been raised about the direct association between the number of pollen grains and the concentration of allergenic proteins in the air, responsible for the allergic reaction in people with pollinosis (Buters et al., 2012; Plaza et al., 2016b). The results of the comparison of pollen and allergen concentrations in our work generally indicate an adequate relationship between both measurements, and in any case, the health alert systems can use the levels of pollen exposure in the area of cities as a risk index (Alcázar et al., 2015; Pfaar et al., 2019; Porcel Carreño et al., 2020). In fact, our analysis shows that between 64 and 87 % of the cases depending on the pollen type, are classified within the confidence interval of pollen-allergen regression line. As evidence of this, numerous previous works have related the severity of symptoms in allergic patients with exposure to pollen from allergenic species (Damialis et al., 2019; Feo Brito et al., 2010; Jones et al., 2019).

Efforts to explain in detail the allergen load using pollen concentrations are complicated by significant discrepancies between both variables, with different types of particle concentrations and different units of measurement (Moreno-Grau et al., 2006; Plaza et al., 2016a). In this work, we propose a new measurement of the discrepancy between pollen and allergen concentrations – namely the value of the residuals of a linear regression – to characterize the relationship between pollen and allergen (Plaza et al., 2020). We propose this new methodology to define the cases of greatest discrepancy outside the confidence interval that characterises the association between pollen and allergen throughout the whole of the study period; that is, anomalous cases where there is a greater quantity of allergen compared to pollen ('High' cases), or a greater quantity of pollen compared to allergen ('Low' cases).

Environmental patterns have a very complex effect on the relation between pollen and allergens. Interannual variations in the allergen-pollen ratio might be even more influenced by environmental changes than by intrinsic physiological mechanics which makes multi-years studies very interesting. On the one hand, the biological particles recorded at a specific moment depend on the meteorological conditions that have prevailed throughout a series of previous days; and on the other, different meteorological variables may exert a dissimilar influence on the aerobiological process of each particle, pollen or allergen (Cresti and Linskens, 2000; Rojo et al., 2015).

The release of pollen grains from the anther is favoured by high temperature and low relative humidity and the influence of meteorological factors continues during the dispersal process. Although the primary carriers for pollen allergens are intact pollen grains, the presence of free aeroallergens could explain the discrepancies between pollen and allergen concentrations. There are different reasons why allergens can be free in the atmosphere, as the release of starch granules when pollen grains come in contact with water or the rupture of pollen grains in the air after hydration (Aloisi et al., 2018; Cresti and Linskens, 2000; Firon et al., 2012). Therefore, pollen is exposed to the variable environmental conditions in the atmosphere since it is released from the anther. Our results show a clear and gradual response to the meteorological conditions of previous days, and in general, a greater influence of variables such as humidity, temperature and precipitation was observed from the current day up to 6 days prior to the time the pollen and allergens were recorded. However, not all pollen types react in the same way.

Platanus allergen-pollen ratio showed a greater influence of the meteorological variables on the current day and the day before, whereas in the

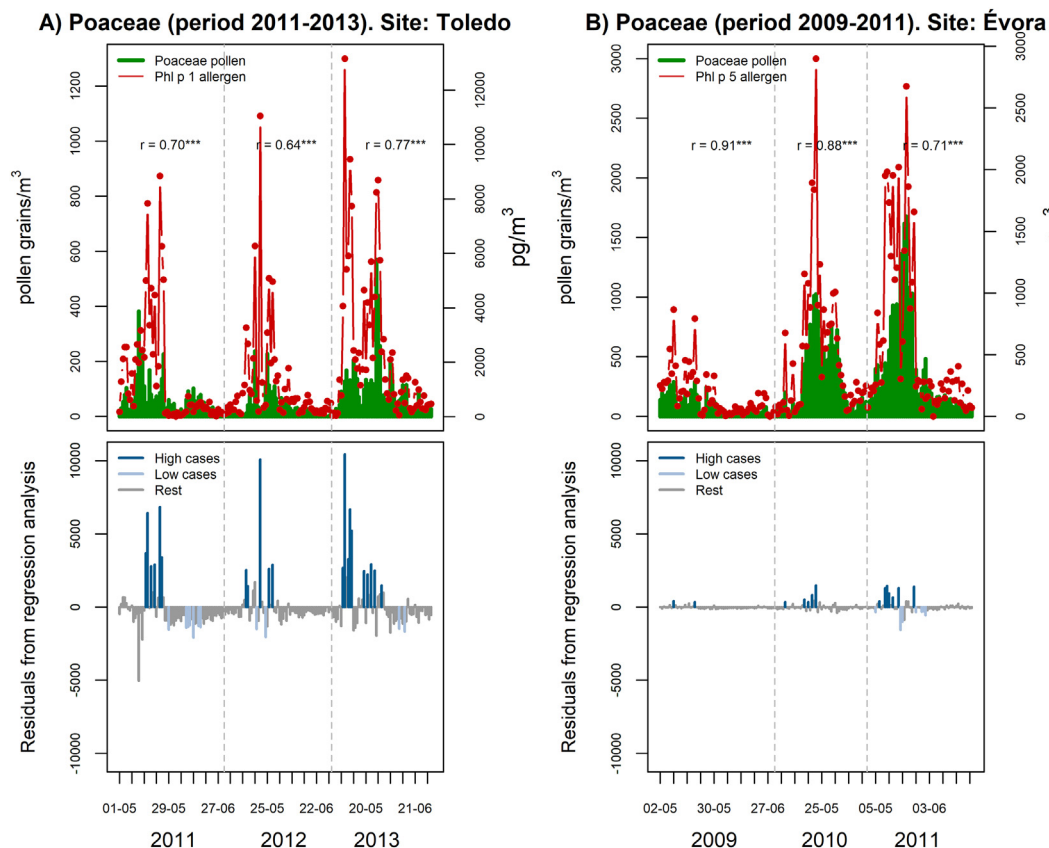


Fig. 4. Top: annual relationship between (A) Poaceae pollen and Phl p 1 concentrations in Toledo (Spain) during 2011–2013, and (B) Poaceae pollen and Phl p 5 concentrations in Évora (Portugal) during 2009–2011. r Spearman correlation test. Bottom: special cases outside the 99 % confidence interval. Value of the residuals is the difference between the observed and the obtained values of the regression analysis from Fig. 1. “High” cases correspond to positive residuals and “Low” cases correspond to negative residuals.

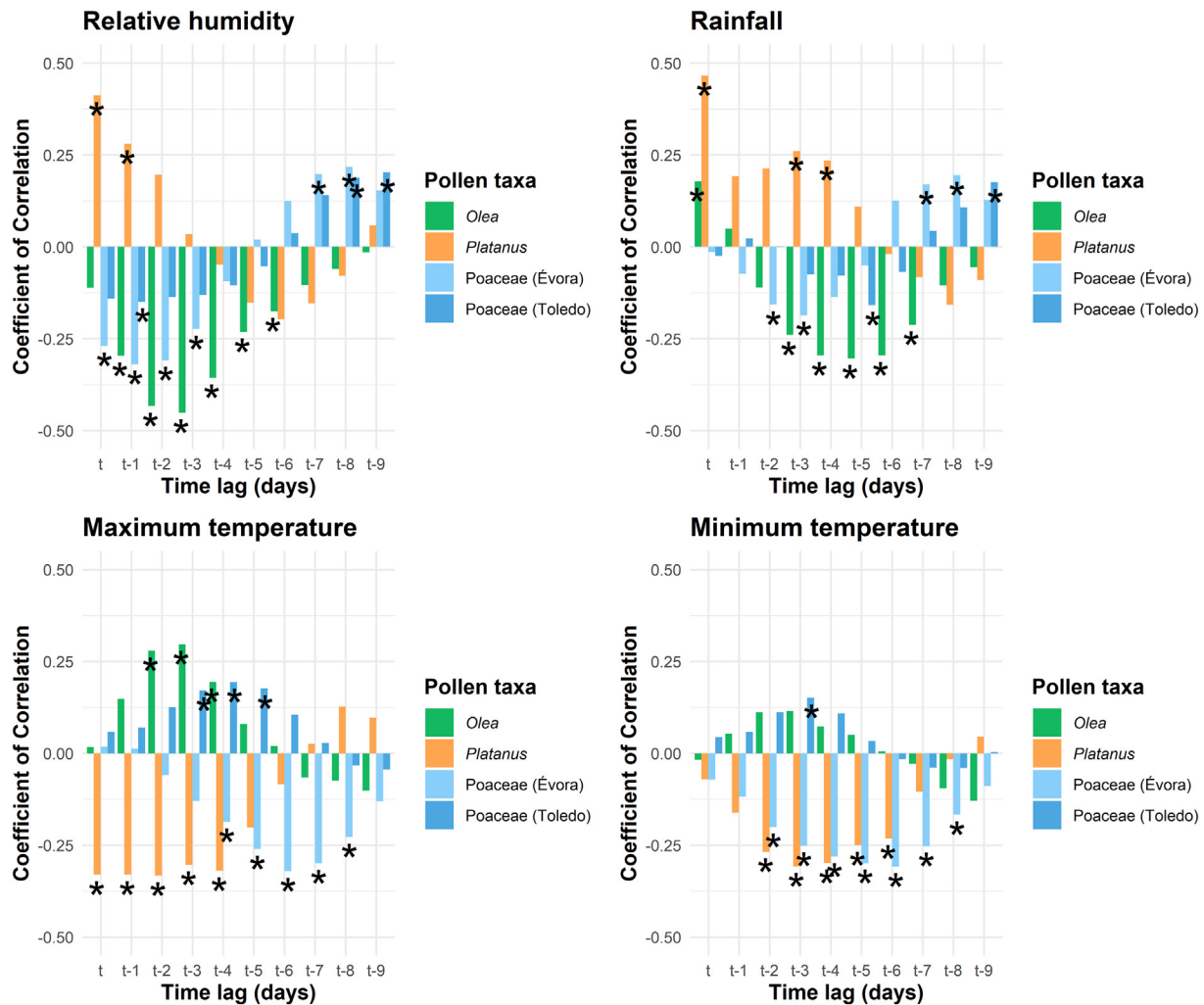


Fig. 5. Spearman's correlation test between all residual data from the regression analysis between pollen and allergens and the random component of meteorological variables considering different time lags (a 3-day moving average was applied).

cases of *Olea* and *Poaceae*, the greatest influence occurred with the meteorological variables for 2 to 4 days prior. In this regard, the origin of the biological particles and the dispersion time until their capture by the samplers is very important. In the case of *Platanus*, the biological particles come from ornamental cultivation that is highly localised within the cities, so the transport time can be assumed to be very short, and the local environmental conditions will have a significant effect (Adams-Groom et al., 2017; Rojo et al., 2019). The opposite occurs with *Olea*. In Toledo, olive cultivation is widely distributed far from the city, and this type of pollen has a high dispersive capacity (Moreno-Grau et al., 2016; Rojo et al., 2016b), so the airborne pollen and aeroallergens are transported for longer in the atmosphere and the greatest influence occurs several days prior to the emission of the pollen.

However, the differences observed between *Platanus* and *Olea* and *Poaceae* not only indicate divergences in the period of greatest influence, but also in the sign of the influence of variables such as relative humidity and temperature, which are different for each type of pollen. In the case of relative humidity, the greatest effect on the allergen of *Platanus* according to our results may occur during the dispersion and deposition of the pollen grain, where an increase in environmental humidity would cause a greater allergen load compared to pollen concentration, as it has been shown for *Platanus* and other pollen types (Buters et al., 2015; Fernández-González et al., 2019). The allergen Pla a 1 is a protein involved in the development of the pollen tube and fecundation that is rapidly released from

the pollen grain under conditions of hydration (Suárez-Cervera et al., 2005). The release effect of relative humidity on the Pla a 1 allergen has been demonstrated through in vitro tests, where the influence of a range of parameters can be analysed separately (Aloisi et al., 2018). However, the complex interaction between environmental variables does not allow this effect to be clearly discerned (Fernández-González et al., 2013).

The pollen types *Olea* and *Poaceae* have shared characteristics that may explain why they show the same relation with relative humidity. The pollen season of *Olea* and *Poaceae* overlaps in time and occurs during May and June when the temperature begins to increase more rapidly, and the relative humidity drops to an average level of 25–50 %. During this period, the influence of meteorological variables may be more closely related with the process of pollen emission, which would be favoured by low humidity and high temperatures (Cresti and Linskens, 2000; D'Amato and Cecchi, 2008; Rojo et al., 2015). These meteorological conditions lead to an increase in allergens, even several days later, and this delay is more evident in the case of *Olea* due to the medium and long distance transport from pollen sources located several dozen kilometres away (Oteros et al., 2015; Rojo et al., 2016a).

The same relation between allergen-pollen ratio and meteorological variables has been found in two very different bioclimatic areas, namely Toledo (Spain) and Évora (Portugal). The similarity of the pattern observed with relative humidity and precipitation is also particularly important, as

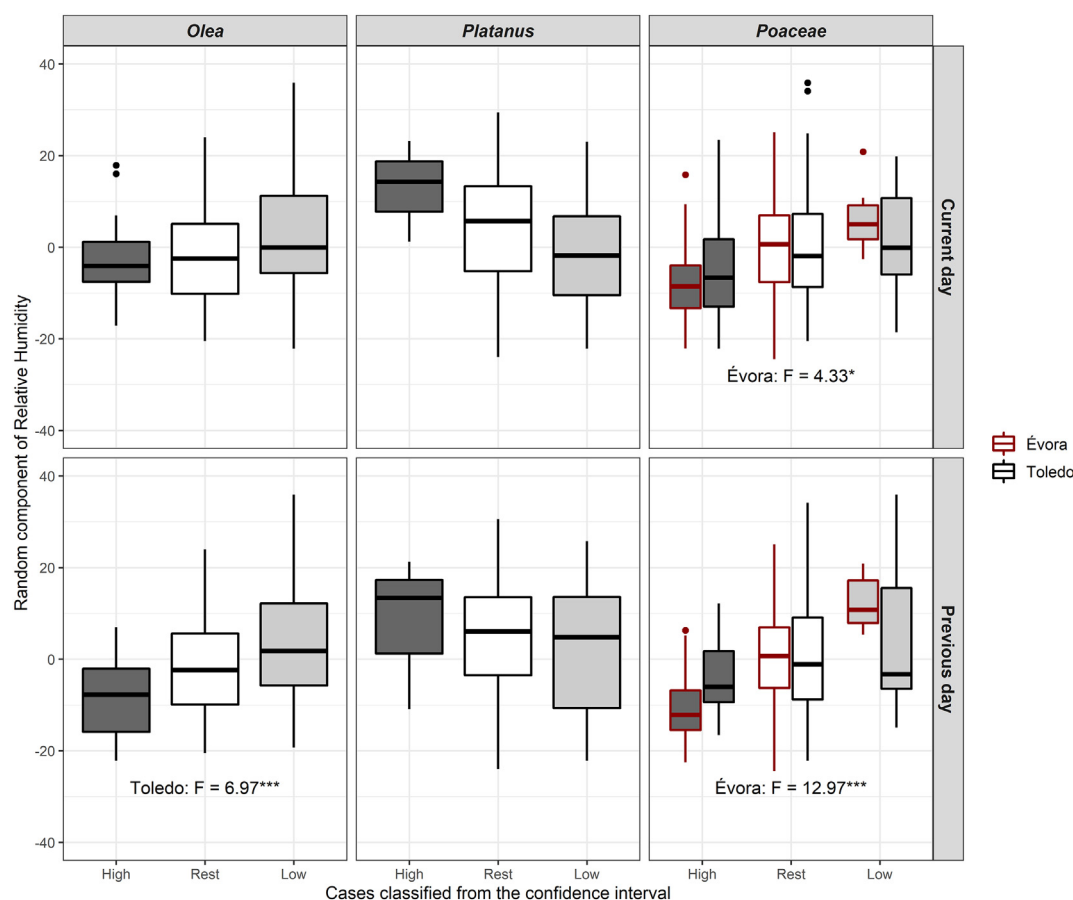


Fig. 6. Effect of the anomalies (residual) of the Relative Humidity variable with respect to the residual data from the regression analysis between pollen and allergens for the current and the previous day. “High” cases represent more allergen concentrations and “Low” cases represent less allergen concentrations than the normal allergen-pollen ratio (linear regression, Fig. 1).

a different system was used to capture the particulate matter in both cases, although as it has been demonstrated by Plaza et al. (2017), both capture systems are adequate for the quantification of allergens in the air. In the light of these results, we believe that the findings observed in relation to Poaceae pollen and allergens present considerable robustness regardless of location, period or sampling system. However, the different allergen of grasses considered in both locations (Phl p 1 and Phl p 5) should be taken in account as a limitation to carry out an intercomparison of allergenic potency of pollen and allows only to compare in parallel the observed environmental patterns in both cases.

5. Conclusions

Although our results support the relationship between aeroallergen load and airborne pollen concentrations, it is important to gain a greater understanding of the relation between both measurements in regard to environmental patterns. In this way, the results of this work allow us to interpret the rate of discrepancy between allergens and pollen by studying the patterns of meteorological variables up to several days beforehand and they can be used in allergy risk prediction models generated from atmospheric pollen.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.159630>.

Authors' contributions

Conceptualization and design methodology: Jesus Rojo, Celia M. Antunes, and Rosa Pérez-Badia.

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Data interpretation: all authors.

Writing and editing of the manuscript: all authors.

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Final approval of the manuscript: all authors.

Data availability

The authors do not have permission to share data.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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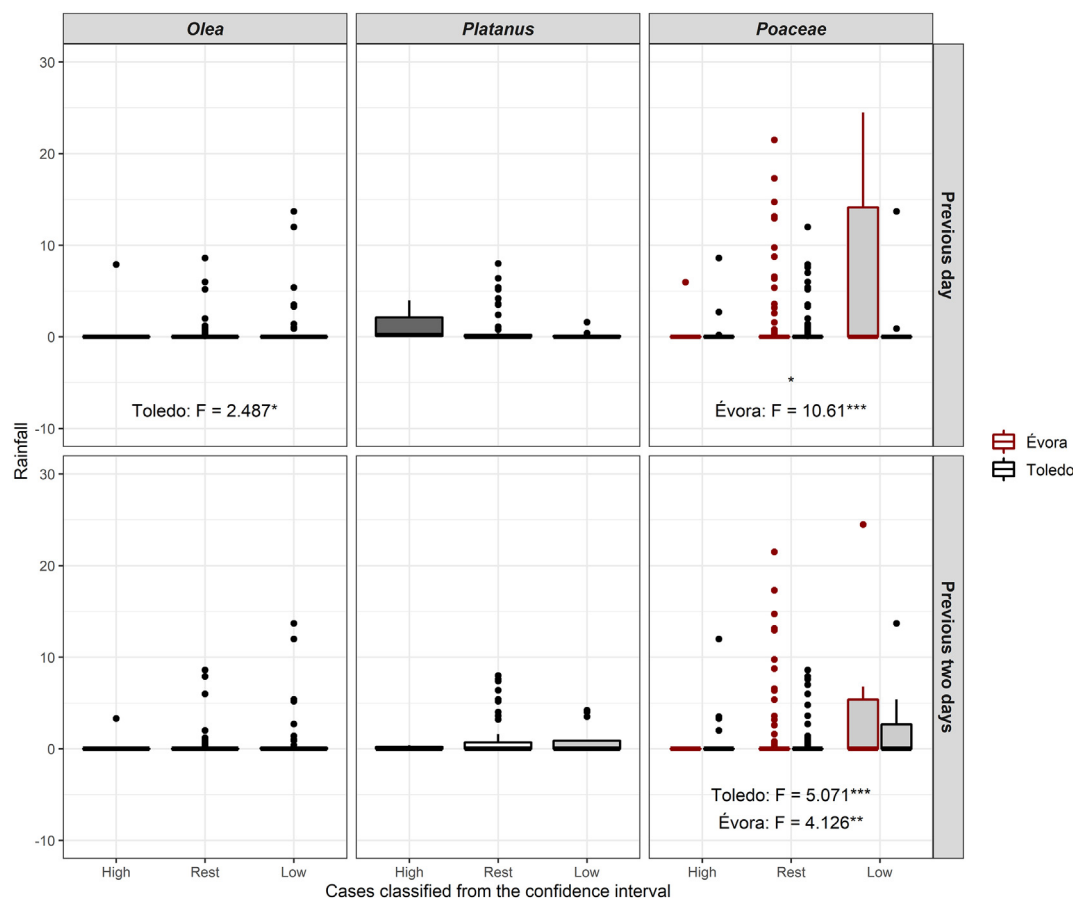


Fig. 7. Effect of Rainfall variable with respect to the residual data from the regression analysis between pollen and allergens for the current and the previous day. “High” cases represent more allergen concentrations and “Low” cases represent less allergen concentrations than the normal allergen-pollen ratio (linear regression, Fig. 1).

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References

- Adams-Groom, B., Skjøth, C.A., Baker, M., Welch, T.E., 2017. Modelled and observed surface soil pollen deposition distance curves for isolated trees of *Carpinus betulus*, *Cedrus atlantica*, *Juglans nigra* and *Platanus acerifolia*. *Aerobiologia* (Bologna) 33, 407–416. <https://doi.org/10.1007/s10453-017-9479-1>.
- AEMET, 2022. Guía resumida del clima en España 1981-2010. Retrieved from Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, Madrid. www.aemet.es.
- Alcázar, P., Galán, C., Torres, C., Domínguez-Vilches, E., 2015. Detection of airborne allergen (Pla a 1) in relation to platanus pollen in Córdoba, South Spain. *Ann. Agric. Environ. Med.* 22, 96–101. <https://doi.org/10.5604/12321966.1141376>.
- Alché, J., Castro, A.J., Olmedilla, A., Fernández, M., Rodríguez, R., Villalba, M., Rodríguez-García, I., 1999. The major olive pollen allergen (Ole e 1) shows both gametophytic and sporophytic expression during anther development, and its synthesis and storage takes place in the RER. *J. Cell Sci.* 112, 2501–2509.
- Aloisi, I., Del Duca, S., De Nuntiis, P., Vega Maray, A.M., Mandrioli, P., Gutiérrez, P., Fernández-González, D., 2018. Behavior of profilins in the atmosphere and in vitro, and their relationship with the performance of airborne pollen. *Atmos. Environ.* 178, 231–241. <https://doi.org/10.1016/j.atmosenv.2018.01.051>.
- Andersson, K., Lidholm, J., 2003. Characteristics and immunobiology of grass pollen allergens. *Int. Arch. Allergy Immunol.* 130, 87–107. <https://doi.org/10.1159/000069013>.
- 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. Allergy Asthma Immunol.* 89, 83–89. [https://doi.org/10.1016/S1081-1206\(10\)61916-3](https://doi.org/10.1016/S1081-1206(10)61916-3).
- Arilla, M.C., Ibarrola, I., Mir, A., Monteseirín, J., Conde, J., Martínez, A., Asturias, J.A., 2005. Development of a Sandwich-type ELISA for measuring pla a 1, the major allergen of *Platanus acerifolia* pollen. *Int. Arch. Allergy Immunol.* 138, 127–133. <https://doi.org/10.1159/000088434>.
- Asam, C., Hofer, H., Wolf, M., Aglas, L., Wallner, M., 2015. Tree pollen allergens - an update from a molecular perspective. *Allergy Eur. J. Allergy Clin. Immunol.* <https://doi.org/10.1111/all.12696>.
- Asturias, J.A., Ibarrola, I., Bartolome, B., Ojeda, I., Malet, A., Martínez, A., 2002. Purification and characterization of pla a 1, a major allergen from *Platanus acerifolia* pollen. *Allergy* 57, 221–227. <https://doi.org/10.1034/j.1398-9995.2002.03406.x>.
- Barnes, C.S., 2018. Impact of climate change on pollen and respiratory disease. *Curr Allergy Asthma Rep* 18, 59. <https://doi.org/10.1007/s11882-018-0813-7>.
- Bastl, K., Kmenta, M., Geller-Bernstein, C., Berger, U., Jäger, S., 2015. Can we improve pollen season definitions by using the symptom load index in addition to pollen counts? *Environ. Pollut.* 204, 109–116. <https://doi.org/10.1016/j.envpol.2015.04.016>.
- Buters, J., Prank, M., Sofiev, M., Pusch, G., Albertini, R., Annesi-Maesano, I., Antunes, C., Behrendt, H., Berger, U., Brandao, R., Celenk, S., Galan, C., Grewling, L., Jackowiak, B., Kennedy, R., Rantio-Lehtimäki, A., Reese, G., Sauliene, I., Smith, M., Thibaudon, M., Weber, B., Cecchi, L., 2015. Variation of the group 5 grass pollen allergen content of airborne pollen in relation to geographic location and time in season. *J. Allergy Clin. Immunol.* 136, 87–95.e6. <https://doi.org/10.1016/j.jaci.2015.01.049>.
- Buters, J.T.M., Antunes, C., Galveias, A., Bergmann, K.C., Thibaudon, M., Galán, C., Schmidt-Weber, C., Oteros, J., 2018. Pollen and spore monitoring in the world. *Clin. Transl. Allergy* 8, 9. <https://doi.org/10.1186/s13601-018-0197-8>.
- Buters, J.T.M., Thibaudon, M., Smith, M., Kennedy, R., Rantio-Lehtimäki, A., Albertini, R., Reese, G., Weber, B., Galan, C., Brandao, R., Antunes, C., Jäger, 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, 496–505. <https://doi.org/10.1016/j.atmosenv.2012.01.054>.
- Cariñanos, P., Casares-Porcel, M., 2011. Urban green zones and related pollen allergy: a review. Some guidelines for designing spaces with low allergy impact. *Landsc. Urban Plan.* 101, 205–214. <https://doi.org/10.1016/j.landurbplan.2011.03.006>.
- Cecchi, L., D'Amato, G., Ayres, J.G., Galan, C., Forastiere, F., Forsberg, B., Gerritsen, J., Nunes, C., Behrendt, H., Akdis, C., Dahl, R., Annesi-Maesano, I., 2010. Projections of the effects of climate change on allergic asthma: the contribution of aerobiology. *Allergy* 65, 1073–1081. <https://doi.org/10.1111/j.1398-9995.2010.02423.x>.
- Cleveland, R.B., Cleveland, W.S., McRae, J.E., Terpenning, I., 1990. STL: a seasonal-trend decomposition procedure based on loess. *J. Off. Stat.* 6 (1), 3–73. <https://doi.org/10.2307/1345502>.
- Cresti, M., Linskens, H.F., 2000. Pollen-allergy as an ecological phenomenon: a review. *Plant Biosyst.* 134, 341–352. <https://doi.org/10.1080/11263500012331350495>.
- D'Amato, G., Cecchi, L., 2008. Effects of climate change on environmental factors in respiratory allergic diseases. *Clin. Exp. Allergy* 38, 1264–1274. <https://doi.org/10.1111/j.1365-2222.2008.03033.x>.
- D'Amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., Liccardi, G., Popov, T., van Cauwenberge, P., 2007. Allergic pollen and pollen allergy in Europe. *Allergy* 62, 976–990. <https://doi.org/10.1111/j.1398-9995.2007.01393.x>.

- Damialis, A., Häring, F., Gökkaya, M., Rauer, D., Reiger, M., Bezold, S., Bounas-Pyros, N., Eyerich, K., Todorova, A., Hammel, G., Gilles, S., Traidl-Hoffmann, C., 2019. Human exposure to airborne pollen and relationships with symptoms and immune responses: indoors versus outdoors, circadian patterns and meteorological effects in alpine and urban environments. *Sci. Total Environ.* 653, 190–199. <https://doi.org/10.1016/j.scitotenv.2018.10.366>.
- De Linares, C., Alcázar, P., Valle, A.M., Díaz de la Guardia, C., Galán, C., 2019. Parietaria major allergens vs pollen in the air we breathe. *Environ. Res.* 176, 108514. <https://doi.org/10.1016/j.envres.2019.05.045>.
- Duffort, O., Quintana, J., Ipsen, H., Barber, D., Polo, F., 2008. Antigenic similarity among group 1 allergens from grasses and quantitation ELISA using monoclonal antibodies to phl p 1. *Int. Arch. Allergy Immunol.* 145, 283–290. <https://doi.org/10.1159/000110887>.
- Esch, R.E., 2004. Grass pollen allergens. *Clin. Allergy Immunol.* 18, 185–205. <https://doi.org/10.3109/9781420061987-12>.
- European Academy of Allergy and Clinical Immunology (EAACI) Advocacy Manifesto Tackling the Allergy Crisis in Europe-Concerted Policy Action Needed.
- Feo Brito, F., Gimeno, P.M., Carnés, J., Fernández-Caldas, E., Lara, P., Alonso, A.M., García, R., Guerra, F., 2010. Grass pollen, aeroallergens, and clinical symptoms in Ciudad Real, Spain. *J. Investig. Allergol. Clin. Immunol.* 20, 295–302.
- Fernández-González, M., Guedes, A., Abreu, I., Rodríguez-Rajo, F.J., 2013. Pla a 1 aeroallergen immunodetection related to the airborne platanus pollen content. *Sci. Total Environ.* 463–464, 855–860. <https://doi.org/10.1016/j.scitotenv.2013.06.062>.
- Fernández-González, M., Ribeiro, H., Pereira, J.R.S., Rodríguez-Rajo, F.J., Abreu, I., 2019. Assessment between platanus pollen and pla a 1 allergen in two cities of North-Western Iberian Peninsula. *Aerobiologia (Bologna)* 35, 463–475. <https://doi.org/10.1007/s10453-019-09576-0>.
- Firon, N., Nepi, M., Pacini, E., 2012. Water status and associated processes mark critical stages in pollen development and functioning. *Ann. Bot.* 109, 1201–1214. <https://doi.org/10.1093/aob/mcs070>.
- Galán, C., Smith, M., Thibaudon, M., Frenguelli, G., Oteros, J., Gehrig, R., Berger, U., Clot, B., Brandao, R., 2014. Pollen monitoring: minimum requirements and reproducibility of analysis. *Aerobiologia (Bologna)* 30, 385–395. <https://doi.org/10.1007/s10453-014-9335-5>.
- García-Mozo, H., 2017. Poaceae pollen as the leading aeroallergen worldwide: a review. *Allergy Eur. J. Allergy Clin. Immunol.* <https://doi.org/10.1111/all.13210>.
- Gökkaya, M., Damialis, A., Nussbaumer, T., Beck, I., Bounas-Pyros, N., Bezold, S., Amisi, M.M., Kolek, F., Todorova, A., Chaker, A., Aglas, L., Ferreira, F., Redegeld, F.A., Brunner, J.O., Neumann, A.U., Traidl-Hoffmann, C., Gilles, S., 2020. Defining biomarkers to predict symptoms in subjects with and without allergy under natural pollen exposure. *J. Allergy Clin. Immunol.* 146, 583–594. <https://doi.org/10.1016/j.jaci.2020.02.037> e6.
- Hirst, J.M., 1952. An automatic volumetric spore trap. *Ann. Appl. Biol.* 39, 257–265.
- IPMA, 2022. Instituto Português do Mar e da Atmosfera. Normais climatológicas 1971–2000. Retrieved from www.ipma.pt.
- Jones, N.R., Agnew, M., Banic, I., Grossi, C.M., Colón-González, F.J., Plavec, D., Goodess, C.M., Epstein, M.M., Turkalj, M., Lake, I.R., 2019. Ragweed pollen and allergic symptoms in children: results from a three-year longitudinal study. *Sci. Total Environ.* 683, 240–248. <https://doi.org/10.1016/j.scitotenv.2019.05.284>.
- Lake, I.R., Jones, N.R., Agnew, M., Goodess, C.M., Giorgi, F., Hamaoui-Laguel, L., Semenov, M.A., Solomon, F., Storkey, J., Vautard, R., Epstein, M.M., 2017. Climate change and future pollen allergy in Europe. *Environ. Health Perspect.* 125, 385–391. <https://doi.org/10.1289/EHP173>.
- Lara, B., Rojo, J., Fernández-González, F., Pérez-Badia, R., 2019. Prediction of airborne pollen concentrations for the plane tree as a tool for evaluating allergy risk in urban green areas. *Landscape Urban Plan.* 189, 285–295. <https://doi.org/10.1016/j.landurbplan.2019.05.002>.
- Maya-Manzano, J.M., Fernández-Rodríguez, S., Monroy-Colín, A., Silva-Palacios, I., Tormo-Molina, R., Gonzalo-Garijo, Á., 2017. Allergenic pollen of ornamental plane trees in a Mediterranean environment and urban planning as a prevention tool. *Urban For. Urban Green.* 27, 352–362. <https://doi.org/10.1016/j.ufug.2017.09.009>.
- Moreno-Grau, S., Aira, M.J., Elvira-Rendueles, B., Fernández-González, M., Fernández-González, D., García-Sánchez, A., Martínez-García, M.J., Moreno, J.M., Negral, I., Vara, A., Rodríguez-Rajo, F.J., 2016. Assessment of the olea pollen and its major allergen ole e 1 concentrations in the bioaerosol of two biogeographical areas. *Atmos. Environ.* 145, 264–271. <https://doi.org/10.1016/j.atmosenv.2016.09.040>.
- Moreno-Grau, S., Elvira-Rendueles, B., Moreno, J., García-Sánchez, A., Vergara, N., Asturias, J.A., Arilla, M.C., Ibarrola, L., Seoane-Camba, J.A., Suárez-Cervera, M., 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. Allergy Asthma Immunol.* 96, 858–864. [https://doi.org/10.1016/S1081-1206\(10\)61350-6](https://doi.org/10.1016/S1081-1206(10)61350-6).
- Oteros, J., García-Mozo, H., Alcázar, P., Belmonte, J., Bermejo, D., Boi, M., Cariñanos, P., Díaz de la Guardia, C., Fernández-González, D., González-Minero, F., Gutiérrez-Bustillo, A.M., Moreno-Grau, S., Pérez-Badia, R., Rodríguez-Rajo, F.J., Ruiz-Valenzuela, L., Suárez-Pérez, J., Trigo, M.M., Domínguez-Vilches, E., Galán, C., 2015. A new method for determining the sources of airborne particles. *J. Environ. Manag.* 155, 212–218. <https://doi.org/10.1016/j.jenvman.2015.03.037>.
- Pawankar, R., Walter Canonica, G., Holgate, S.T., Lockey, R.F., Blaiss, M.S., 2013. *World Allergy Organization (WAO). White Book on Allergy: Update 2013*.
- Pérez-Badia, R., Rapp, A., Morales, C., Sardinero, S., Galán, C., García-Mozo, H., 2010. Pollen spectrum and risk of pollen allergy in Central Spain. *Ann. Agric. Environ. Med.* 17, 139–151.
- Pfaar, O., Karatzas, K., Bastl, K., Berger, U., Buters, J., Darsow, U., Demoly, P., Durham, S., Galán, C., Gehrig, R., Gerth van Wijk, R., Jacobsen, L., Katsifarakis, N., Klimek, L., Saarto, A., Sofiev, M., Thibaudon, M., Werchan, B., Bergmann, K.C., 2019. Pollen season is reflected on symptom load for grass and birch pollen-induced allergic rhinitis in different geographic areas - an EAACI Task Force Report. *Allergy*, all.14111 <https://doi.org/10.1111/all.14111>.
- Plaza, M.P., Alcázar, P., Galán, C., 2016a. Correlation between airborne Olea europaea pollen concentrations and levels of the major allergen ole e 1 in Córdoba, Spain, 2012–2014. *Int. J. Biometeorol.* 60, 1841–1847. <https://doi.org/10.1007/s00484-016-1171-6>.
- Plaza, M.P., Alcázar, P., Hernández-Ceballos, M.A., Galán, C., 2016b. Mismatch in aeroallergens and airborne grass pollen concentrations. *Atmos. Environ.* 144, 361–369. <https://doi.org/10.1016/j.atmosenv.2016.09.008>.
- Plaza, M.P., Alcázar, P., Oteros, J., Galán, C., 2020. Atmospheric pollutants and their association with olive and grass aeroallergen concentrations in Córdoba (Spain). *Environ. Sci. Pollut. Res.* 27, 45447–45459. <https://doi.org/10.1007/s11356-020-10422-x>.
- Plaza, M.P., Alcázar, P., Velasco-Jiménez, M.J., Galán, C., 2017. Aeroallergens: a comparative study of two monitoring methods. *Aerobiologia (Bologna)* 33, 363–373. <https://doi.org/10.1007/s10453-017-9475-5>.
- Porcel Carreño, S., Gómez Nieves, E., Fernández-Caldas, E., Abel Fernández, E., Cases, B., Tudela, J., Maghfour Martin, Y., Domínguez Domínguez, E., Alvarado Arenas, M., Jiménez Timón, S., Ahmida, T., García Ponce, J., Jiménez Gallardo, P., Alvarado Izquierdo, M., Hernández Arbeiza, J., 2020. Immunochemical and physical quantitation of grass and olive pollen allergens: correlation with asthma admissions in Cáceres, Spain. *J. Investig. Allergol. Clin. Immunol.* 30, 334–339. <https://doi.org/10.18176/jiaci.0434>.
- R Core Team, 2019. R: a language and environment for statistical computing. Retrieved from R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Reinmuth-Selzle, K., Kampf, C.J., Lucas, K., Lang-Yona, N., Fröhlich-Nowoisky, J., Shiraiwa, M., Lakey, P.S.J., Lai, S., Liu, F., Kunert, A.T., Ziegler, K., Shen, F., Sgarbanti, R., Weber, B., Bellingshausen, L., Saloga, J., Weller, M.G., Duschl, A., Schuppan, D., Pöschl, U., 2017. Air pollution and climate change effects on allergies in the anthropocene: abundance, interaction, and modification of allergens and adjuvants. *Environ. Sci. Technol.* 51, 4119–4141. <https://doi.org/10.1021/acs.est.6b04908>.
- Rivas-Martínez, S., Penas, Á., del Río, S., González, T.E.D., Rivas-Sáenz, S., 2017. *Bioclimatology of the Iberian Peninsula and the Balearic Islands. The Vegetation of the Iberian Peninsula*. Springer, pp. 29–80.
- Rojo, J., Orlandi, F., Pérez-Badia, R., Aguilera, F., Ben Dhia, A., Bouziane, H., Díaz de la Guardia, C., Galán, C., Gutiérrez-Bustillo, A.M., Moreno-Grau, S., Msallem, M., Trigo, M.M., Fornaciari, M., 2016a. Modeling olive pollen intensity in the Mediterranean region through analysis of emission sources. *Sci. Total Environ.* 551–552, 73–82. <https://doi.org/10.1016/j.scitotenv.2016.01.193>.
- Rojo, J., Oteros, J., Pérez-Badia, R., Cervigón, P., Ferencova, Z., Gutiérrez-Bustillo, A.M., Bergmann, K.C., Oliver, G., Thibaudon, M., Albertini, R., Rodríguez-De la Cruz, D., Sánchez-Reyes, E., Sánchez-Sánchez, J., Pessi, A.-M., Reinharju, J., Saarto, A., Calderón, M.C., Guerrero, C., Berra, D., Bonini, M., Chiodini, E., Fernández-González, D., García, J., Trigo, M.M., Myszkowska, D., Fernández-Rodríguez, S., Tormo-Molina, R., Damialis, A., Kolek, F., Traidl-Hoffmann, C., Severova, E., Caeiro, E., Ribeiro, H., Magyar, D., Makra, L., Udvardy, O., Alcázar, P., Galán, C., Borycka, K., Kasprzyk, I., Newbigin, E., Adams-Groom, B., Apangu, G.P., Frisk, C.A., Skjøth, C.A., Radišić, P., Šikoparija, B., Celenk, S., Schmidt-Weber, C.B., Buters, J., 2019. Near-ground effect of height on pollen exposure. *Environ. Res.* 174, 160–169. <https://doi.org/10.1016/j.envres.2019.04.027>.
- Rojo, J., Rapp, A., Lara, B., Fernández-González, F., Pérez-Badia, R., 2015. Effect of land uses and wind direction on the contribution of local sources to airborne pollen. *Sci. Total Environ.* 538, 672–682. <https://doi.org/10.1016/j.scitotenv.2015.08.074>.
- Rojo, J., Rapp, A., Lara, B., Sabariego, S., Fernández-González, F., Pérez-Badia, R., 2016b. Characterisation of the airborne pollen spectrum in Guadalajara (central Spain) and estimation of the potential allergy risk. *Environ. Monit. Assess.* 188, 130. <https://doi.org/10.1007/s10661-016-5129-2>.
- Ščevková, J., Vašková, Z., Šepšiová, R., Kováč, J., 2021. Seasonal variation in the allergenic potency of airborne grass pollen in Bratislava (Slovakia) urban environment. *Environ. Sci. Pollut. Res.* 28, 62583–62592. <https://doi.org/10.1007/s11356-021-15179-5>.
- Sénéchal, H., Visez, N., Charpin, D., Shahali, Y., Peltre, G., Biolley, J.-P., Lhuissier, F., Couderc, R., Yamada, O., Malrat-Domenge, A., Pham-Thi, N., Poncet, P., Sutra, J.-P., 2015. A review of the effects of major atmospheric pollutants on pollen grains, pollen content, and allergenicity. *Sci. World J.* 2015, 1–29. <https://doi.org/10.1155/2015/940243>.
- Smith, P.M., Ong, E.K., Knox, R.B., Singh, M.B., 1994. Immunological relationships among group I and group V allergens from grass pollen. *Mol. Immunol.* 31, 491–498. [https://doi.org/10.1016/0161-5890\(94\)90068-X](https://doi.org/10.1016/0161-5890(94)90068-X).
- Suárez-Cervera, M., Asturias, J.A., Vega-Maray, A., Castells, T., López-Iglesias, C., Ibarrola, I., Arilla, M.C., Gabarayeva, N., Seoane-Camba, J.A., 2005. The role of allergenic proteins pla a 1 and pla a 2 in the germination of Platanus acerifolia pollen grains. *Sex. Plant Reprod.* 18, 101–112. <https://doi.org/10.1007/s00497-005-0002-4>.
- Takahashi, Y., Ohashi, T., Nagoya, T., Sakaguchi, M., Yasueda, 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 (Bologna)* 17, 313–318.
- Tegart, L.J., Johnston, F.H., Borchers Arriagada, N., Workman, A., Dickinson, J.L., Green, B.J., Jones, P.J., 2021. 'Pollen potency': the relationship between atmospheric pollen counts and allergen exposure. *Aerobiologia* 37, 825–841. <https://doi.org/10.1007/s10453-021-09726-3>.
- Tummon, F., Lucas, Arboledas A., Bonini, M., Guinot, Benjamin, Hicke, M., Jacob, C., Kendrovski, V., Mc Cairns, W., Petermann, E., Peuch, V.-H., Pfaar, Oliver, Sicard, Michaël, Sikoparija, B., Clot, Bernard, 2021. The need for Pan-European automatic pollen and fungal spore monitoring: a stakeholder workshop position paper. *Copernicus Atmos. Monit. Serv.* <https://doi.org/10.1002/clt2.12015>.
- Vara, A., Fernández-González, M., Aira, M.J., Rodríguez-Rajo, F.J., 2016. Fraxinus pollen and allergen concentrations in Ourense (South-Western Europe). *Environ. Res.* 147, 241–248. <https://doi.org/10.1016/J.ENVRES.2016.02.014>.
- Visez, N., Chassard, G., Azarkan, N., Naas, O., Sénéchal, H., Sutra, J.-P., Poncet, P., Choël, M., 2015. Wind-induced mechanical rupture of birch pollen: potential implications for allergen dispersal. *J. Aerosol Sci.* 89, 77–84. <https://doi.org/10.1016/j.jaerosci.2015.07.005>.
- Visez, N., Ivanovsky, A., Roose, A., Gosselin, S., Sénéchal, H., Poncet, P., Choël, M., 2019. Atmospheric particulate matter adhesion onto pollen: a review. *Aerobiologia* 9. <https://doi.org/10.1007/s10453-019-09616-9>.