

Article



# London Plane Tree Pollen and Pla A 1 Allergen Concentrations Assessment in Urban Environments

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Abstract: The London plane tree is frequently used in gardens, parks, and avenues in European urban areas for ornamental purposes with the aim to provide shade, and given its tolerance to atmospheric pollution. Nevertheless, unfortunately, over recent decades, bioaerosols such as Platanus pollen grains cause increasing human health problems such as allergies or respiratory tract infections. An aerobiological sampling of airborne Platanus pollen and Pla a 1 allergen was performed using two volumetric traps placed on the roof of the Science Faculty building of the city of Ourense from 2009 to 2020. A volumetric sampler Hirst-type Lanzoni VPPS 2000 (Lanzoni s.r.l. Bologna, Italy) was used for pollen sampling. Pla a 1 aeroallergen was sampled by using a Burkard Multi-Vial Cyclone Sampler (Burkard Manufacturing Co., Ltd., Hertfordshire, UK) and by means of the enzyme-linked immunosorbent assay (ELISA) technique. Data mining algorithms, C5.0 decision trees, and rulebased models were assessed to evaluate the effects of the main meteorological factors in the pollen or allergen concentrations. Plane trees bloom in late winter and spring months in the Northwestern Spain area. Regarding the trends of the parameters that define the Platanus pollen season, the allergen values fitted the concentrations of pollen in the air in most cases. In addition, it was observed that a decrease in maximum temperatures causes a descent in both pollen and allergen concentrations. However, the presence of precipitations only increases the level of allergens. When the risk of allergy symptomatology was jointly assessed for both the concentration of pollen and allergens in the study area, the number of days with moderate and high risk for pollen allergy in sensitive people increased with respect to traditional alerts considering only the pollen values.

Keywords: Platanus sp.; Pla a 1; pollen; urban environment; C5.0 decision trees algorithm

# 1. Introduction

Aerobiological studies can be used as a tool to detect transformations in plant communities during a given period of time [1]. The adaptation of plants to the different intervention processes such as extensive deforestation (whether massive or selective), the extraction of firewood or wood, the establishment of agricultural or livestock uses and their associated transformations, and the effect of climate change, could change the composition of landscapes [2,3]. Under the aforementioned conditions, the less tolerant plant components of vegetal diversity could be supressed by other plant components that now characterize a large part of the biodiversity of European countries in general, and of the Mediterranean in particular [4]. The analysis of long-term aerobiological data also reflects the influence of the climatic characteristics of the area on the plant species of a certain geographical region, obtaining information on the adaptation of plant communities to changes in climatic conditions as well as possible variations in the duration and intensity of pollination [1]. Therefore, the airborne pollen content can be used as effective bioindicators of the impacts of climate change, since the advance or delay of phenological events is widely considered for the study of global climate change [2,3].



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). *Platanus hispanica Miller* Ex Münchh, also known as London plane tree or *Platanus x acerfolia*, a hybrid of *Platanus occidentalis* L. and *Platanus orientalis* L. [5–7], is a long-lived anemophilous tree frequently used in gardens, parks, and avenues in the European urban areas [8,9] for ornamental purposes with the aim to provide shade [10–14]. In addition, plane trees are often planted in cities due to their tolerance to atmospheric pollution [15]. However, unfortunately, over recent decades, bioaerosols such as *Platanus* pollen grains cause increasing human health problems such as allergies or infections [16]. Nowadays, type I allergic diseases such as rhinoconjunctivitis, atopic eczema, and bronchial asthma have become a global problem that can affect up to 40% of the population in industrialized countries [17]. In addition, several studies reported that there is a higher prevalence of pollen-related sensitization cases in urban environments than in rural areas [18,19].

The *Platanus* pollen incidence of respiratory diseases has been recognized by many authors [10,20–24]. In Central and Southern European cities, sensitization to *Platanus* pollen represents a recognized problem [10,15,24–27]. Some studies suggested that 8%–9% of allergy sensitization rates can be attributed to the plane tree pollen in South European cities, such as Ourense and Santiago de Compostela [11], and 52%–56% in Madrid [19,20].

Regarding respiratory diseases, knowledge of the pollen load in the air in a given geographical area that could cause allergic reactions in sensitive people represents the most valuable information for starting treatment administration. Exposure to allergens represents a key factor for symptomatology [27]. In the case of *Platanus*, two proteins have been described as major and specific allergens, Pla a 1 and Pla a 2 [28,29]. Pla a 1 is a non-glycosylated protein with a molecular weight of 18 kDa, which has a prevalence of 92% in monosensitized patients allergic to *Platanus* and 83% in polysensitized patients [28]. Pla a 2 is a 43 kDa glycoprotein with a prevalence of 83% [28,29]. In addition, a minor allergen, Pla a 3, which belongs to non-specific lipid transport proteins and is an aeroallergen related to food allergy, was identified [30–33]. The cross-reactivity between *Platanus* and other pollen species such as *Artemisia*, *Betula*, *Cupressus*, Chenopodiaceae, *Olea*, *Parietaria*, *Plantago*, and Poaceae was noted by several studies [12,20,28,34,35]. Moreover, studies conducted by several authors reported that people sensitized to *Platanus* pollen may suffer cross-reactivity with some edible vegetables such as lettuce, celery, peach, banana, apple, or hazelnut [11,14,35–39].

The planting and introduction of non-native ornamental vegetation in urban areas imply the appearance of new respiratory allergens, which causes major changes in the immunological response and an increase in cross-reactivity between pollen and food [40]. The plane tree represents about 12% of the total annual air pollen in Ourense, registering a short flowering period with an intense release of pollen into the atmosphere. The aim of our study was to quantify the airborne *Platanus* pollen and their major aeroallergen Pla a 1 content as a source of pollution in Ourense, as well as to evaluate its relationship and the influence of the main meteorological parameters. This information could help us to predict the real allergenic load in the air to prevent possible periods of allergenic risk for sensitized people.

#### 2. Materials and Methods

#### 2.1. Study Area

The study was carried out from 2009 to 2020 in the Ourense city  $(42^{\circ}20'41.4'' \text{ N}-7^{\circ}51'19.87'' \text{ W})$ , located in the North-western of the Iberian Peninsula, in the bioclimatic Mediterranean region (Figure 1). The area is described as oceanic with Mediterranean features, with an annual mean temperature of 14 °C, and 772 mm of annual total precipitation [41].



**Figure 1.** Sampling location area Ourense (red dot) and the surrounding land use by CORINE land cover.

## 2.2. Aerobiological Monitoring

Aerobiological sampling of airborne *Platanus* pollen and Pla a 1 allergen was performed using two volumetric traps placed on the roof of the Science Faculty building, at a height of 15m above the ground level and near the town centre. A volumetric sampler Hirst-type Lanzoni VPPS 2000 (Lanzoni s.r.l. Bologna, Italy) [42] was used for pollen sampling. The device was working continuously with a suction flow rate of 10 L/min simulating the human breathing. Pollen grains were captured using a Melinex tape coated with a 2% silicone solution. Next, the samples were mounted on glass slides and the pollen was quantified and identified using an optical microscope at 40x magnification, applying the method proposed by the Spanish Aerobiology Network (REA) [43]. Pollen data were expressed as average daily pollen grains per cubic meter of air when referring to daily mean values, or pollen integral for total values [44]. The Main Pollen Season (MPS) is considered as the period where the 95% of the annual total pollen is recorded, starting the day when the accumulated sum of pollen reaches the 2.5% to the date when 97.5% is registered [45]. To calculate the risk thresholds for sensitization because of allergenic proteins, a regression analysis was performed that correlated daily pollen data with aeroallergen concentrations [46].

Pla a 1 aeroallergen was sampled by using a Burkard Multi–Vial Cyclone Sampler (Burkard Manufacturing Co. Ltd. Hertfordshire, U.K.) with a 16.5 L/min of aspiration flow rate. The sampler was also located on the roof of the Science Faculty building, next to the pollen trap. The Pla a 1 proteins were collected directly into 1.5 mL Eppendorf vials every 24h. Finally, in order to calculate the Pollen Allergen Potency (PAP) we make the ratio between airborne pollen counts and allergen concentrations [47].

#### 2.3. Enzyme-Linked Immunosorbent Assay (ELISA) Technique

The Pla a 1 samples collected into 1.5 mL Eppendorf vials were analyzed following the method proposed by [48] with some modifications [49]. After centrifugation at 13,400× g rpm for 3 min, dry samples were stirred with 120 µL of extraction buffer that contained 150 mM/L NaCl, 125 mM/L ammonium bicarbonate, 3 mM/L EDTA and 0.005% Tween 20, for 2 h at room temperature. Then, the extract was separated from the particulate matter by centrifugation at 4000× g rpm for 10 min and stored in pellet form at -20 °C. Aeroallergen content in the bioaerosol was quantified using a specific 2-site ELISA methodology [50,51]. Microtiter plates were coated with a specific monoclonal antibody (5D4 at 0.5 µg) in phosphate-buffered saline solution (PBS) and incubated overnight at room temperature in a moist chamber. Coated wells were blocked (200 µL/well) with PBS–BSA–T, which contained 1% bovine serum albumin and 0.05% Tween 20, and incubated for 1 h at 37 °C. Then, the plates were incubated for 1 h at 37 °C with purified Pla a 1 (100  $\mu$ L/well) from a stock of natural Pla a 1 in PBS–BSA–T while the extracted airborne samples are added too (100  $\mu$ L/well). Afterward, the plates were incubated for 1 h at 37 °C with biotinylated rabbit anti-Pla a 1 polyclonal antibody with 625 ng/mL (200  $\mu$ L/well). Later, the plates were incubated for 1 h at 37 °C with streptavidin-conjugated peroxidase in PBS–BSA–T (100  $\mu$ L/well). Then, wells were incubated at room temperature in the dark with a solution of o–phenylenediamine, the substrate for the enzyme AF. Finally, the reaction was stopped after 30 min by adding 3M H<sub>2</sub>SO<sub>4</sub>, and the absorbance was measured at 492 nm. The standard curve was constructed from ten points using a four-parameter logistic curve fit. The three antibodies used in the present study, monoclonal antibody Pla a 1, natural antibody Pla a 1, and biotinylated antibody Pla a 1, were provided by Bial Industrial Farmacéutica, Spain. The daily concentrations were transformed to nanograms per cubic meter of air in order to be compared with the pollen data.

#### 2.4. Meteorological Data

The meteorological data considered for the study were maximum, minimum, and average temperatures (°C), relative humidity (%), rainfall (mm), wind speed (m/s), and wind direction (°). The data were supplied by the "Ourense" and "Ourense–station" meteorological stations of MeteoGalicia, placed 400 m. from the pollen and allergen samplers.

Figure 2 shows the number of days the wind blew in different directions in each study year during the main pollen season. In general, the prevailing winds came from the SW for several days, but in 2009, 2012, and 2013 the winds came from the NE (Figure 2). So, the wind direction pattern does not influence the pollen concentrations.



**Figure 2.** Wind rose of Ourense, number of days along the main pollen season in each study year in that wind blows in each direction.

## 2.5. Statistical Analysis

#### 2.5.1. Correlation Analysis

To calculate the number of days with risk of allergy, the pollen thresholds followed by allergists from the Spanish Society of Allergology and Clinical Immunology (SEAIC) in our study area were considered [52]. The risk threshold for *Platanus* was established in three categories: low (<50 grains/m<sup>3</sup>), moderate (50–130 grains/m<sup>3</sup>), and high (>130 grains/m<sup>3</sup>) [52]. Moreover, a regression equation was carried out between the pollen and allergen data to assess a categorization of the aeroallergens concentrations that correspond with the aforementioned

pollen thresholds. The obtained aeroallergen thresholds were also applied to calculate the number of days with potential hazards of allergy.

The association and effects between the pollen or allergen concentrations and the meteorological factors during the main pollen season were analyzed using Spearman's non-parametric correlation test, setting confidence intervals at 99% (p < 0.01) and 95% (p < 0.05). In addition, a second degree regression equation was performed between the pollen and allergen data during the pollen season to obtain an equation to calculate aeroallergen concentrations that correspond to the aforementioned pollen thresholds. The aeroallergen thresholds obtained were also applied to calculate the number of days with potential allergy risk. The IBM SPSS Statistics version 25.0 package New York, NY, USA was used for the statistical analysis.

## 2.5.2. Data Mining Algorithm: C5.0 Decision Trees and Rule-Based Models

The "C5.0 Decision Trees and Rule-Based Models" algorithm (C5.0 package version 0.1.4.) for R software 4.0.2. [53] was applied to further analyze the relationship between aerobiological and meteorological data. This is a data mining procedure for data exploration and identification of unknown patterns [54]. The C5.0 is one of the most widely used algorithms, which builds models to predict or identify the class or type to which belongs an element, based on the values of entry or explanatory variables [55]. Among the numerous data mining methods, the C5.0 decision trees and rule-based models belong to the supervised classification methods. These methods attempt to determine the relationship between input attributes (explanatory or independent variables) and a target attribute (dependent variable), a relationship represented by a model structure. Models usually describe and explain hidden phenomena in the dataset and can be used to predict the value of the target attribute by knowing the values of the attributes or input variables [56]. In the decision tree construction, the C5.0 algorithm uses the information gain as the standard to obtain the best grouping variables and the cut-off point for the classification procedure, considering the size of the information gain and the cost of obtaining this information [57]. In addition, the algorithm version used in the present work (C5.0) has a great improvement with respect to the previous version (C4.5), since the current version includes the boosting meta-algorithm that reduces bias and variance in a supervised machine learning context. In C5.0, boosting generates a predetermined number of classifiers (decision trees) instead of just one, enhancing these "weak" classifiers to achieve a higher degree of success, leading to a "strong" classifier. Boosting in C5.0 version increases the accuracy of the decision tree model [58].

The data considered for the application of the algorithm were the meteorological, pollen, and allergen concentrations during the main pollen season (MPS) of the studied years (2009–2020). Daily aeroallergen concentrations were considered as the dependent variable, which was transformed into a qualitative variable, and the weather variables were considered as independent explanatory variables. For the characterization of the allergenicity thresholds of aeroallergens, the results of the regression analysis that correlates daily pollen data against aeroallergen load were applied, as a result of this analysis we have generated two allergen risk categories. For the training data set, 80% of the daily data of all the variables considered were considered (randomly selected by means of the "set.seed" function). For the validation data set, the remaining 20% of the cases were considered in order to verify whether the obtained model was not overfitted by the training data, as well as to verify the model's performance [45]. Once this step was completed, a new model was obtained using the entire available dataset.

To determine the accuracy of the model in each case, a confusion matrix was computed to obtain the percentage of successful predictions vs. actual classification data, considering the aforementioned thresholds (moderate and high aeroallergen levels). The confusion matrix crosses the observed (real class) and predicted values (data obtained after applying the algorithm) in a double input table. The accuracy of the model was obtained from the relation between correct predictions and the total number of samples, following the equation:

Accuracy = 
$$(TP + TN)/(TP + FN + FP + TN)$$

where:

- TP: true positive
- TN: true negative
- FN: false negative
- FP: false positive.

#### 3. Results

Plane trees bloom in late winter and spring months in the Northwestern Spain area. *Platanus* pollen season lasted less than a month, with an average duration of 29 days during the study period (2009–2020), starting in the second half of March and ending in the second half of April. However, some oscillations were recorded in the start date of the MPS, since it could be delayed to the month of April in 2018 and advanced to the first ten days of March in 2020 (Table 1). The end MPS date was delayed to May some years (2013, 2018). Over the study period, the Annual Pollen Integral (APIn) registered an average of 5927 pollen grains as well as 6.020 nanograms of Pla a 1 allergen (Table 1).

**Table 1.** Start and end date and length (days) of the main pollen season (MPS), annual pollen integral (APIn) during the MPS (pollen), mean of pollen (pollen/m<sup>3</sup>), pollen peak (pollen/m<sup>3</sup>), and pollen peak day (day), allergen concentration during MPS (ng), mean of allergen (ng/m<sup>3</sup>), allergen peak (ng/m<sup>3</sup>), allergen peak day (day) and pollen allergen potency (PAP) (ng/pollen).

	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2009-2020
Start (day)	18-Mar	30-Mar	25-Mar	19-Mar	28-Mar	20-Mar	28-Mar	21-Mar	16-Mar	6-Apr	21-Mar	7-Mar	22-Mar
End (day)	14-Apr	24-Apr	14-Apr	9-Apr	5-May	23-Apr	17-Apr	24-Apr	20-Apr	8-May	13-Apr	28-Mar	19-Apr
Length (days)	28	26	21	22	39	35	21	35	36	33	24	22	29
APIn (pollen)	4401	4664	4069	8452	4252	7160	9848	3909	7290	5399	5128	6552	5927
Mean (pollen/m <sup>3</sup> )	157	179	203	384	109	205	469	115	203	164	214	298	225
Peak (pollen/m <sup>3</sup> )	900	1970	992	2347	1138	921	2323	438	1549	932	747	1644	1325
Peak date (day)	19-Mar	6-Apr	29-Mar	22-Mar	30-Mar	20-Mar	30-Mar	29-Mar	29-Mar	6-Apr	29-Mar	11-Mar	26-Mar
Allergen (ng)	7.765	9.437	0.521	4.791	1.293	6.365	3.361	5.300	13.927	5.170	2.180	12.125	6.020
Mean (ng/m <sup>3</sup> )	0.311	0.363	0.025	0.218	0.033	0.182	0.160	0.156	0.387	0.157	0.091	0.551	0.219
Peak $(ng/m^3)$	0.893	1.458	0.109	0.840	0.134	0.369	0.277	0.477	1.157	0.535	0.279	1.239	0.647
Peak date (day)	19-Mar	7-Apr	28-Mar	28-Mar	29-Mar	25-Mar	2-Apr	30-Mar	21-Mar	15-Apr	29-Mar	19-Mar	28-Mar
PAP (ng/pollen)	0.0018	0.0020	0.0001	0.0006	0.0003	0.0009	0.0003	0.0014	0.0019	0.0010	0.0004	0.0019	0.0010

The highest values of annual pollen integral were recorded during the year 2015 with 9848 pollen grains, while the maximum allergenic load was reached in 2017 with 13.927 ng (Table 1). The maximum daily pollen peak in all years of the study was recorded on 22 March 2012 with 2347 pollen/m<sup>3</sup>, while the daily allergen peak was registered on 7 April 2010 with 1.458 ng/m<sup>3</sup> (Table 1). Both peaks coincided with a period of rainfall absence and increased temperatures, and the allergen peak was produced after precipitations during the previous days (Table 1, Figure 3).

Regarding the trends of the parameters that define the *Platanus* pollen season, the allergen values fitted the concentrations of pollen in the air. In addition, it was observed that a decrease in maximum temperatures causes a descent in both pollen and allergen concentrations. However, in some cases these two parameters do not match at all, for example in presence of precipitations only the level of allergens increases (Figure 3). The pollen allergen potency (PAP) index, which is considered the rate between the allergen and pollen concentrations, was calculated. The highest value was 0.0020 ng/pollen registered in the year 2010, and the lowest was recorded in 2011 with 0.0001 ng/pollen (Table 1).



**Figure 3.** Pollen grain concentrations (grey area), allergen concentrations (black line), maximum temperature (dash line), and rainfall (bars) for *Platanus* during the main pollen season on each study year.

To assess the effects of the main meteorological parameters in the *Platanus* airborne pollen and Pla a 1 allergen content, a Spearman correlation test was conducted (Figure 4). Taking into account the whole data set period during the MPS, the obtained results showed the higher significant degree of association between the pollen and the allergen concentrations. A negative significant correlation with the minimum temperature was observed for both pollen and allergen (Figure 4). A positive correlation with a 99% of significance (p < 0.01) was obtained with average and maximum temperatures in three of the studied years (2009, 2012, 2019). A negative correlation with a 95% of significance (p < 0.05) was observed with minimum temperature in the year 2015. Overall, relative humidity presented a significant negative correlation and rainfall recorded a similar effect on pollen and allergen concentration. Finally, a significant positive correlation was obtained with wind speed in the year 2010 (Figure 4).



■ Average temperature ■ Maximum temperature ■ Minimum temperature ■ Relative humidity 🖾 Rainfall 🖸 Wind speed ■ Pollen

**Figure 4.** Spearman correlations between pollen (**A**) or allergen (**B**) and the main meteorological variables in each year and all study years. The dotted line means that above the line the significance level is p < 0.01.

The risk allergy periods to *Platanus* were calculated considering the number of days in which the pollen values exceeded the potential risk thresholds proposed by SEAAIC [52]. On average, 7 days a year the moderate allergy risk was exceeded, and for 11 days the high risk. A great number of moderate hazard days was registered in 2014 and 2017 years, while 2014 was the year that showed major number of days with a higher risk (Table 2).

Table 2. Allergy risk periods for pollen and allergen thresholds.

	Thresholds (I	Pollen/m <sup>3</sup> )	1	Thresholds (ng/m <sup>3</sup> )				
mod 51-	erate ·130	high >130		moderate 0.279–0.463	high >0.463			
Risk (days)								
	Polle	en	Alle	ergen	Pollen and allergen			
	moderate	high	moderate	high	moderate	high	days	
2009	3	10	3	9	6	19	25	
2010	8	7	5	9	13	16	29	
2011	4	11	0	0	4	11	15	
2012	4	11	0	7	4	18	22	
2013	9	7	0	0	9	7	16	
2014	11	15	9	0	20	15	35	
2015	5	13	0	0	5	13	18	
2016	10	10	10	1	20	11	31	
2017	11	13	6	11	17	24	41	
2018	4	13	6	2	10	15	25	
2019	5	11	1	0	6	11	17	
2020	5	13	0	12	5	25	30	
Average 2009–2020	7	11	3	4	10	15	25	

Regression equation was performed to identify aeroallergen thresholds for moderate and high risk of symptom onset in sensitized individuals. The model obtained used allergen content as the dependent variable and pollen concentration as the independent variable and showed an adjust  $R^2$  of 0.790 (Figure 5).



**Figure 5.** *Platanus* pollen concentrations (pollen/ $m^3$ ) versus allergen values ( $ng/m^3$ ) during the main pollen season in the study years.

With this regression equation, we established an aeroallergen threshold based on the pollen thresholds above described. Two categories were generated, "moderate" when aeroallergen value ranging between 0.279 and 0.463 ng/m<sup>3</sup> and "high" when the allergen value is higher than 0.463 ng/m<sup>3</sup>. Considering the allergen data, the moderate risk threshold was exceeded for an average of 3 days a year and in the case of high risk 4 days. The year that recorded the greatest number of days with moderate potential risk was 2016, while 2020 in the case of the allergenic risk of high potential hazard (Table 2). When the threat of allergy symptomatology was jointly assessed for both, pollen and allergen concentrations, our results showed an average of 10 days per year under moderate risk threshold and 15 days of high risk for pollen allergy. The highest number days of moderate and high risk were recorded in 2014, 2016 and 2020 respectively (Table 2).

Finally, Data Mining C5.0 decision tree algorithms were applied for the relationship of pollen or aeroallergens with meteorological factors. A decision tree was obtained for Pla a 1 allergens, but an inexact decision tree model was found for *Platanus* pollen. The Pla a 1 allergens model identified six terminal nodes. In each terminal node, the homogeneity in the classification of the elements belonging to each class (high and moderate allergen level) and the purity of each node are observable.

The Pla a 1 C5.0 model developed from the entire data set combines three variables, the minimum temperature (MinT) applied to 100% of the cases, the relative humidity (RH) to 37.87% of the cases, and the maximum temperature (MaxT) in 23.67% of cases. Three of the six terminal nodes were classified as a moderate allergen, nodes 4, 7, and 11 with 10.42% and 17.39% and 100% of cases classified in this group, respectively. The other three terminal nodes were classified as a high allergen (with a higher purity degree in these nodes), showing values of 42.86% of cases classified in this group in node 2, 40.00% of cases in node 8, and 28.57% of cases in node 10 (Figure 6).



**Figure 6.** C5.0 model for Pla a 1 allergen developed from the 2009–2020 dataset. Terminal nodes indicate the classification of cases based on percentage represented in dark grey (moderate pollen) or light grey (high pollen).

The accuracy of the developed C5.0 model was tested as the percentage of correct prediction in the classification of cases comparing the real and predicted data in a confusion matrix (Table 3). The percentage of success was calculated by the sum of the cases on the diagonal of the matrix divided by the sum of all the elements of the matrix. The results did not show an overfitting of the model to the training data set, since when we apply the algorithm to the validation data set, we obtain a prediction percentage of 58.33%. Finally, we used the entire dataset for the development of a new model obtaining a higher accuracy percentage of 66.57%.

Table 3. Confusion matrix applied to determine Pla a 1 model accuracy.

		Observed Class			
		high-allergen	moderate-allergen		
ed Class		Correctly classified	Misclassified		
	high-allergen	high allergen cases	cases		
		141	100		
Predict		Misclassified	Correctly classified		
	moderate-allergen	cases	moderate allergen cases		
		13	84		

# 4. Discussion

It is widely known that several atmospheric biological particles, including pollen grains, cause problems in human health such as allergies and infections [15]. The incidence of *Platanus* pollen allergy varies depending on the flowering period. Regarding the pollen season, *Platanus* is an arboreal species with spring flowering in different countries such as China [59,60], the United Kingdom [61], and France [62]. As in other spring species, the climatic conditions of the months prior to this period determine the annual plane trees flowering [63–65]. In our study, the pollen season took place on average from 22 March to 19 April. An intense and short blooming period, reaching high concentrations in a very short period of 29 days in total was observed, according to the notes in several studies [10,11,23,24,66]. Previous studies conducted by our research group about the influence of climatic change on *Platanus* pollination in different bioclimatic areas across

Europe reveal the plane tree as a good bioindicator of temperature variations [65]. An increase in temperature was detected when the continentally of the area increases, and the timing of plane flowering reflects this trend of air temperature with a delay of their

more concentrated and with a peak value very easily identifiable [65]. The allergenic importance of *Platanus* lies in the large amount of pollen it emits and in the abundance of its trees in cities [66]. Nevertheless, the annual pollen integral (APIn) concentrations and the daily pollen peaks varied considerably between years. Among the possible causes that justify these differences may be the more frequent growth of the specimens and the implementation of pruning strategies [5,12,67]. Pruning is a process that consists of cutting off branches of shrubs and trees [68] used to optimize fruit production as a management tool in agricultural, forestry, and ornamental species [69]. However, this cultural practice could also be applied in cities to limit the growth of tree inflorescences, reducing the induction of flower buds and the number of inflorescences and, therefore, the concentrations of pollen in the air [12,70]. Selective pruning just before the flowering season prevents the definitive formation of inflorescences, which reduces pollen grains and the allergenic load of the environment [69–71]. Studies conducted by [13] reported a significant decrease in pollen emissions produced by more directed pruning before flowering. In the city of Ourense, the pruning policy for ornamental trees began to change from 2016. This fact was reflected in our study as the annual pollen integral was reduced around 1000 pollen/m<sup>3</sup> by year, with the most pronounced differences in 2016 and 2019. Adequate pruning and management strategies before flowering reduce the risk of allergens during the flowering period, although it would affect the accuracy of prediction models.

flowering. Lower temperatures determine a release of *Platanus* pollen in the atmosphere

Traditionally, the concentration of pollen grains in the atmosphere was the information used to prevent sensitive people [10]. Nevertheless, several studies have reported over the past few years that the period of symptoms in sensitive people often does not coincide with the season of pollen exposure [10,23-27,72-74]. The allergenic proteins have a much smaller size than pollen grains facilitating their penetration into the bronchi [75–77]. This fact also enables their release of pollen and anthers during the periods of previous and subsequent flowering periods and pollen peaks [78]. This fact could be the main cause of the discrepancies between the allergen and pollen peaks [79], that explain episodes of patient's symptomatology in periods with low pollen concentrations. In addition, some researchers noted that rain events before the pollen peak lead to humid conditions that result in protein release from deposited dry pollen, increasing allergenic risk [80,81]. In our study, the precipitations registered before the pollen peak could explain the discordances between the content of pollen and allergens in the air during the years 2014, 2018, and 2020, mainly in 2018, since a strong storm before the pollen peak induced a higher allergens release during this weather episode. Several studies noted the appearance of protein peaks simultaneously with precipitations due to the discharge of cytoplasmic material from pollen grains during a thunderstorm [10,82]. Our results showed that the allergen peak was reached after the pollen peak in the years 2011, 2013, and 2017, during a rainy period.

Pollen thresholds have become key information for the administration of the correct treatment in sensitive patients. Pollen threshold levels is a topic that is currently under development. Different authors pointed out that the pollen thresholds for the development of allergic symptoms depend on different factors such as the ethnic population, the variability of the pollen season, the amount of allergens carried in the pollen grains, climatic conditions and air pollutants [83]. The complex relationship between these aforementioned factors makes it difficult to generalize the use of a certain pollen or allergen threshold. In the case of plane tree pollen, the thresholds indicated by different authors are quite similar, with some discrepancies in the case of the pollen concentrations that prompt high allergy risk. The Spanish Society of Allergology and Clinical Immunology (SEAIC) established high levels of 130 grains/m<sup>3</sup> [52] while the Spanish Aerobiology network (REA) pointed out levels higher than 200 grains/m<sup>3</sup> [43]. In the Northern Europe areas, a threshold of 100 grains/m<sup>3</sup> for trees pollen was indicated [84,85]. In order to know the real periods of allergy risk in our

study area, a regression equation was developed to calculate the threshold concentrations of Pla a 1 that correspond to the categories of allergy to pollen marked by the Spanish Society of Allergology and Clinical Immunology (SEAIC) for *Platanus* [52]. An average of 25 days of risk events was observed considering both pollen and aeroallergen throughout the study. In addition, in the years 2010, 2014, 2016, 2017and 2020 the total number of days is greater, especially in the years 2014 and 2017, with 35 and 41 days respectively of pollen and allergenic hazard. In general, in all study years there is a higher risk of high risk due to allergen than due to pollen, except in the years 2011, 2012, 2013, 2014, 2015, 2016, 2018 and 2019, which was the opposite. This evidence could not be found if only pollen were counted.

In addition, quantifying the aeroallergen Pla a 1 is important to avoid cases of false positives, considering that *Platanus* pollen is cross-reactive with pollen allergens from different species, as well as with foods of plant origin. Several studies have shown airborne reactions between *Platanus* and taxa whose pollen release occurs during the same period as Poaceae, *Cupressus, Betula, Olea, Parietaria, Plantago, Artemisia,* and Chenopodiaceae [20,28,34]. Regarding cross-reactivity reactions with plant foods, the statistical association between *Platanus* and fruits such as hazelnuts, peanuts, bananas, and celery is so strong that it leads to the determination that patients allergic to plant foods are a subgroup within allergic patients to pollen [35]. This cross-reactivity is attributed to the non-specific lipid-transfer protein allergen Pla a 3 [86,87]. However, Pla a 2 must also be taken into account, since Pla a 1 has the function of modifying the cell walls of the pollen in the extracellular space, and Pla a 2 is responsible for pollen-stigma adhesion [87]. This biological function implies that during the characteristic autumn precipitations, *Platanus* pollen can be resuspended from the leaves of trees or other surfaces triggering reactions in sensitized people [88].

Considering the main meteorological parameters, a high dependence on temperature has been observed in plants with spring and early summer flowering [89], such as Platanus, being more influenced by warmer winters and springs and, therefore, presenting an earlier start of the pollen season [63,89,90], as well as an extended length and higher pollen load [89,91,92]. Furthermore, urban environments suffer from elevated temperatures and different moisture availability compared to surrounding rural areas. The urban heat island effect can influence plant phenology and thus the main characteristics of the pollen season. A larger urban pavement surface increases the temperature in cities and decreases the water retention capacity, which will lead to greater alterations of the current phenological variations. In addition, climate change processes could also intensify the magnitude of the variations, since the increase in temperature causes advances in the start date of flowering and delays in the end date, while the reduction in accumulated rainfall could affect pollen production [93,94]. The analysis of the main meteorological variables, *Platanus* pollen and allergen concentrations varied throughout the study years because of interannual climatic differences. When we analyze year by year, Spearman's correlation analysis showed a high positive correlation between pollen content and allergen concentration. In general, no correlations were obtained between pollen and allergens, and wind speed throughout the study years, which could be a consequence of the fact that pollen and allergens in the atmosphere of the study area are not transported, and they come from the arboreal populations of the city. Only a high positive and significant correlation between pollen and allergen with wind speed was recorded in 2010 with a predominantly SW wind direction in agreement with the findings reported for the city of Thessaloniki [95]. In contrast, a high negative correlation between pollen or allergen with minimum temperature (2015, 2016, and 2019 years), relative humidity (2009, 2012, 2015, and 2019 years), and rainfall (2009, 2015, and 2019 years). When the study years set was analyzed (2009–2020), it was observed that the meteorological factor with a high degree of negative association was the minimum temperature. Several authors have reported similar results in the same study area [11,27,96]. On the other hand, studies across Europe show how temperature contributes to seasonal changes such as pollen load or season length [5,10,13,27,97,98]. This occurs in the present study in those years with low maximum temperatures and, therefore, with longer MPS, where the degree of association was negative with the maximum and mean temperature. Seasonal changes are expected to increase in the future due to the temperature dependency of plane trees and the estimation of future temperature increases of up to 1.5 °C between 2030 and 2052 [99].

Finally, the C5.0 model developed for the aeroallergen Pla a 1 coincides with the Spearman correlation test in the variables used. The model used minimum temperature, relative humidity, and maximum temperature as classifiers, with this order of relevance and a marked predominance of minimum temperature, and with six terminal nodes, three of them high purity and three moderate purity. The first division was applied by the minimum temperature below 7.6 °C that generate node 2 of high allergen, with 42.86% of purity. If this value is exceeded, the decision tree continues to branch, after using the relative humidity with a cut-off point below 66.5% (node 4 with a moderate allergen). On the contrary, when the relative humidity exceeds 66.5% the decision tree continues to branch and subsequently uses the maximum temperature with a cut-off point of 21.4 °C. When the maximum temperature is below 21.4 °C the decision tree uses the relative humidity with one break point of 84.8%. When relative humidity is below the breaking point, node 7 will be a moderate allergen. On the contrary, if the relative humidity is higher than 84.8% the resulting terminal node is a high allergen (node 8). However, if the maximum temperature exceeds 21.4 °C the decision tree uses the minimum temperature with a cut-off point of 12.7 °C with two terminal nodes taking place. Node 10 is classified as a high allergen, with 28.57% purity when the minimum temperature is lower than the cut-off point, or if the temperature is higher than 12.7 °C the classification ends on a moderate allergen node (node 11). Therefore, this model showed the relevance of minimum, and maximum temperature and relative humidity in the occurrence of different allergen concentration levels. This behavior can be explained because plants with spring phenology can show an intense response to temperature changes, with early flowering species such as *Platanus* being the most sensitive to this variable [100]. This influence of weather variables on *Platanus* pollen was reported by several studies in different areas of Spain such as Jaen [101], Valladolid [13], Granada [5], Ourense, and Cartagena [10,27]. In the case of the Pla a 1 aeroallergen, the influence of these weather variables was pointed out by several authors in different areas of the Northwest Iberian Peninsula such us Ourense [10,26] or in Ourense and Porto [23].

#### 5. Conclusions

The results of our study confirmed that the combination of pollen counts and allergen quantification should be evaluated to assess the actual biological contamination in the atmosphere, as well as the exposure of the allergic population. When the risk of allergy symptomatology was jointly assessed for both the concentration of pollen and allergens in the area of study, the number of days with moderate and high risk for pollen allergy in sensitive people increased with respect to traditional alerts considering only the pollen values. In addition, our results show that the planning of green areas must follow aerobiological criteria to avoid the use of anemophilous species and make adequate use of pruning.

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