



Cross-reactivity between the Betulaceae family and fallout in the real atmospheric aeroallergen load

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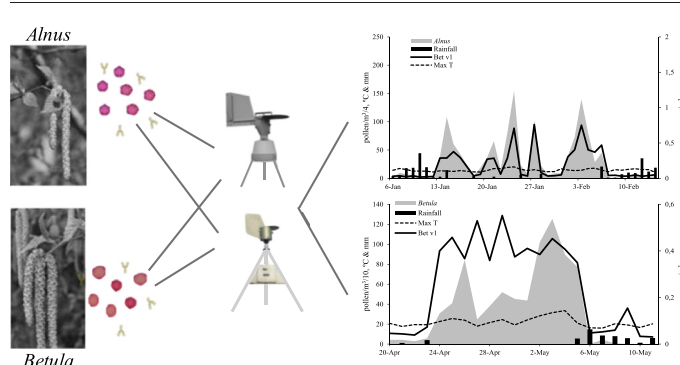
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HIGHLIGHTS

- Major innovation was first detection Aln g1 aeroallergens using Bet v1 antibodies.
- This fact verified cross-reactivity between the main allergens of both pollen types.
- Urban sensitized people to *Betula* could suffer symptoms also in winter due to *Alnus*.
- Greater symptoms under low birch pollen values could be detected: priming effect.
- Airborne pollen and allergen data must be combined to know the real allergenic load.

GRAPHICAL ABSTRACT



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ABSTRACT

Betulaceae family is a dominant tree pollen type in the atmosphere at Northwest Spain, being a major cause of allergenic rhinitis or asthma symptoms. *Alnus* pollen cause symptoms in the 9–20% of the total hay fever sufferers mean while the 41.89% of patients present a positive skin-prick-test for *Betula* allergens. Aln g1 and Bet v1 aeroallergens belong to PR-10 protein family and are associated to cross-reactivity processes. Airborne pollen and aeroallergens from *Alnus* and *Betula* were sampled during their Main Pollen Season from 2016 to 2019. Pollen sampling was conducted by means of a Hirst volumetric trap and aeroallergens were sampled using a Multi Vial Cyclone Sampler.

Alnus flowering took place from January to February, with an average duration of 44 days. *Betula* bloom occurred from April to May with an average pollen season of 33 days. The major innovation of our study was the first detection of Aln g1 allergens in the atmosphere by using Bet v1 antibodies. This fact verified the cross-reactivity between the main allergens of *Betula* and *Alnus* pollen. Along the study period, an average of 18 days/year and 14 days/year with high potential risk of allergy due to *Alnus* pollen and allergen respectively, was registered. For *Betula* an average of 16 days/year with high potential risk of allergy due to pollen and 22 days for allergen was registered. The main consequence of the successive bloom of both trees would be the so-called “priming effect”. Urban population sensitized to *Betula* pollen could suffer allergic symptoms during winter (as a consequence of *Alnus*), and in spring with the manifestation of higher symptoms under low birch pollen grain levels in the atmosphere.

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The traditional information to prevent allergies, such as the airborne pollen concentrations, should be combined with the data of aeroallergen to identify the real allergenic load in the atmosphere.

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1. Introduction

Pollen from Betulaceae family (included in order Fagales) and particularly the *Betula* and *Alnus* genus are amongst the major causes of allergic rhinitis and, maybe, asthma symptoms in Northern and Central Europe where they are dominant pollen types in the atmosphere (Smith et al., 2014; Caillaud et al., 2015). In the Northwestern Spain alder is represented by the specie *Alnus glutinosa* L. (Rodríguez-Rajo et al., 2006a) whereas birch is characterized by the *Betula alba* L. (Moreno, 1990) and *Betula pendula* Roth., the last commonly used as ornamental in some cities (Jato et al., 2007). Both trees belong to the riparian forests of this area, as well as *Salix atrocinerea* Brot., *Frangula alnus* subsp. *alnus* Mill. and *Fraxinus angustifolia* Vahl.

The *Alnus* pollen cause hay fever symptoms from January to February in the 9–20% of the total hay fever sufferers in the Northwestern Spain (Arenas et al., 1996; Belmonte et al., 1998) meanwhile the 41.89% of patients present a positive SPT (skin-prick-test) for *Betula alba* L. allergens, being monosensitized the 10.75% (Varela et al., 2003). The major allergen of the alder tree pollen is *Aln g1* with a molecular weight of 18 kDa (WHO/IUIS database: www.allergen.org). This protein has a recognized cross-reactivity degree with other taxa belonging to the Fagaceae and Betulaceae families. The birch major allergen is *Bet v1*, a protein with a molecular weight of 17 kDa consisting of several isoallergens (Swoboda et al., 1995). The *Bet v1* protein has molecular homology with pollen of several plants, such as hazel, alder, hornbeam and hop-hornbeam (Brier et al., 2018). Several authors noted the existence of cross-reactivity reactions between *Bet v1* and pollen extracts of alder, hornbeam, hazel, oak, chestnut and beech (Niederberger et al., 1998; Ipsen and Hansen, 1991; Ipsen et al., 1985; Wuthrich and Straumann, 1997; Heath et al., 2015). Similar proteins to *Bet v1* were also found in some vegetables and fruits, being responsible for pollen-food cross-reaction (Ebner et al., 1995; Vieths et al., 1995; Jäger, 1995).

Aln g1 and *Bet v1* aeroallergens belong to the pathogenesis-related-10 (PR-10) protein family and are associated to cross-reactivity processes (Deifl et al., 2014; Radauer et al., 2008). The main cause of their cross-reactivity is the primary and the tertiary structural characteristics of proteins. Cross-reactivity phenomena take place under an identity sequence higher than the 70% (Ferreira et al., 2004). If protein identity sequence is lower than 50%, is very difficult this process to occur (Aalberse, 2000). Several researches pointed to higher similarity of three-dimensional structures of *Bet v1*-like allergens can induce cross-reactivity (Gajhede et al., 1996; Schirmer et al., 2005; Hauser et al., 2011). This cross-reactivity could be also associated with the so-called pollen food syndrome (PFS), a common allergic disorder induced by a pollen-vegetables cross-reaction. The most clinical manifestation of PFS is oral allergy syndrome (OAS) prompting different symptomatology as the appearance of itching and tingling of the lips, mouth, and throat immediately after eating some fruits and vegetables (Olcese et al., 2019). The most frequent OAS occurs in *Bet v1* sensitized patients when eating fruits from the Rosaceae family, mainly apple (Webber and England, 2010).

In the case of successive flowering periods between different pollen types with cross-reactivity reactions, it should be taken into account the possible "priming effect" phenomena. Pollen exposure to a given species can reduce the subsequent symptomatology threshold triggered by the next species to flower (Ito et al., 2015; De Weger et al., 2011; Caillaud et al., 2012). At Northwest Spain, the *Alnus* Main Pollen Season (MPS) takes place from January to the first days of March (Rodríguez-Rajo et al., 2006b) and the MPS for *Betula* occurs from the last week of

March to April (Cotos-Yáñez et al., 2004; Rodríguez-Rajo et al., 2003). Because of the successive flowering periods of both taxa, it could be assumed that people may elicit stronger symptoms to *Betula* pollen at lower pollen concentrations (Corden and Millington, 1999; Emberlin et al., 1997).

The main objective of the present study was to detect for first time the *Alnus* aeroallergens presence (*Aln g1*) in the atmosphere by means of the use of the *Betula* major aeroallergen (*Bet v1*), based in their possible cross-reaction ability. In addition, the influence of the main weather variables on airborne pollen and allergen concentrations was evaluated in order to identify the variable that exert the highest impact in the presence of both particles in the atmosphere. The obtained results could help us to better understand and prevent the pollinosis clinical symptoms and to adjust the sensitized people treatment.

2. Material and methods

The study was carried out in Ourense (42°0'N7°5'W), located in Northwestern Spain, with an annual mean temperature of 14 °C and 772 mm of annual accumulated rainfall.

Airborne pollen and aeroallergens from *Alnus* and *Betula* were sampled in the atmosphere during their Main Pollen Season from 2016 to 2019. Pollen sampling was conducted by means of a Hirst type (Hirst, 1952) Lanzoni VPPS 2000 volumetric trap working continuously at a suction flow rate of 10 L/min. The sampler was placed on the roof top of the Science Faculty building of Ourense, approximately 15 m above the ground level. In order to count and identify the pollen grains, an optical microscope with a magnification of 40× was used following the method proposed by the Spanish Aerobiology Network (Galán et al., 2007). Pollen data were expressed as pollen grains for annual or seasonal total pollen or Annual Pollen Integral (APIn) and pollen grains per cubic meter of air when referring to daily mean values (Galán et al., 2017). Aeroallergens were sampled using a Multi Vial Cyclone Sampler (Burkard Manufacturing Co Ltd. Hertfordshire, UK). The samples were collected into 1.5 mL Eppendorf (dry condition) vials during the flowering period of each taxa and stored at −20 °C. The daily samples were quantified by Enzyme Linked Immuno-Sorbent Assay (ELISA) following the protocol proposed by Takahashi et al. (2001) and Moreno-Grau et al. (2006) with some modifications. The samples were extracted with 120 µL buffer for 2 h. There were then centrifuged at 4000 rpm during 10 min, to separate the particulate matter, and stored at −20 °C. The quantification of proteins was conducted by using 2-site antibody ELISA as previously described (Arilla et al., 2002, 2006). Microtiter plates were coated with monoclonal antibody over night at 4 °C. The plates were blocked with PBS (pH 7.4), containing 1% bovine serum albumin and 0.05% Tween 20. The plates were subsequently incubated with natural antibody allergens, or extracted airborne samples. In the next step, a secondary biotinylated antibody was added, and 0.25 µg/mL streptavidin-peroxidase (Sigma). All incubations were performed at 37 °C for 1 h, and 3 washes were conducted with PBS-T between successive steps. Finally, the plates were incubated at room temperature in the dark with *o*-phenylenediamine (OPD) (Sigma). The reaction was stopped after 30 min with sulfuric acid (3 M) in order to measure the absorbance at 492 nm. The extracts were assayed as dilution series, and the daily concentrations were interpolated from the linear portion of the standard curve in ng/m³ of air.

The main pollen season (MPS) was calculated using a logistic model developed by Ribeiro et al. (2007) and modified by Cunha et al. (2015). It's based on fitting annually a non-linear logistic regression model to

the daily accumulated curve for each pollen type. The pollen season start and end dates were calculated using a logistic function based on the asymptotes when pollen amounts are stabilized on the beginning and the end of the accumulated curve. A 10% boundary level was considered to delimit the MPS beginning and end for *Alnus* and *Betula*. Data were managed with the “AeRobiology” R package (Development-Core-Team, 2017; Rojo et al., 2019).

For pollen allergenicity characterization, we followed the classification suggested by the REA (Galán et al., 2007), which classify pollen types in four groups, belonging *Alnus* and *Betula* to the third group. For the characterization of aeroallergens allergenicity, we applied regression analysis correlating daily pollen data vs aeroallergen load.

Weather data (maximum, minimum and average temperature, dew point, relative humidity, rainfall and wind speed) were supplied by the Agencia Estatal de Meteorología (AEMet) from the Science Faculty building of Ourense meteorological station, located at 300 m of both samplers.

Statistical analysis was performed by means of a non-parametric Spearman's correlation test (because the data did not adjust to a normal distribution) in order to analyse the relationship between daily pollen and allergen concentrations and the main meteorological variables. The software IBM SPSS Statistics 24.0 was used for the statistical analysis. In addition, a Principal Components and Factor Analysis (PCFA) was conducted by using the STATISTICA software to detect possible structures in the relationship between variables and to classify them according to their influence on dependent variables' variation (Tipping and Bishop, 1999). The advantage of this non-parametric method is its hardness when the number of variables is high but there are a scarce number of observations (Sabatier et al., 1999).

3. Results

The *Alnus* flowering took place from the first ten days of January to the second ten days of February, with an average MPS duration of 44 days. The year with the highest *Alnus* pollen levels was 2018 with 6152 pollen grains, and the lower amount was registered in 2019 with 2097 pollen grains. The pollen peak was observed on January 24th 2018 with 867 pollen/m³. Additionally, the highest total amount of allergen was registered during the year 2017, with 9.085 ng, as well as the allergen peak with 1.868 ng/m³ on February 1st. The lowest *Alnus* allergen concentration was registered in 2019 with 6.223 ng (Table 1, Fig. 1).

Betula flowering occurred from the first days of April to the early May with an average MPS length of 33 days, except in 2019 in which the flowering advanced to the mid-March. As in the case of alder, the

year 2018 registered the highest total amount of pollen with 8312 pollen grains, as well as the pollen peak of the studied period on April 25th with 1283 pollen/m³. The year with lowest *Betula* pollen amount was 2019 with 843 pollen grains. In addition, the highest total amount of Bet v1 allergen was registered in 2019 with 18.836 ng and an allergen peak of 1.460 ng/m³ on March 31th. The lowest total amount of aeroallergen was registered in 2016 with 5.627 ng. For both *Alnus* and *Betula* the maximum pollen and allergen peaks coincided with a period of rainfall absence and an increase on maximum temperature (Table 1, Fig. 1).

The pollen Allergen Potency (AP) for each year and taxa was calculated. AP is the yearly relationship between pollen and allergen concentrations. In the case of *Alnus* the average AP was 0.002 ng/pollen. The years with higher AP were 2017 and 2019 with 0.003 ng/pollen respectively. For *Betula*, the average AP was 0.007 ng/pollen, with the highest AP value reached in 2019 with 0.022 ng/pollen (Table 1).

The number of days under potential risk of allergy in the atmosphere was calculated following the pollen threshold values established by the REA (Spanish Aerobiology Network). A regression equation was conducted in order to calculate the aeroallergens thresholds in the atmosphere considering the categorized pollen threshold values (Table 2, Fig. 2). The adjusted R² values of the equations ranged from 0.832 to 0.156 for *Alnus* and from 0.540 to 0.168 for *Betula*. Taking into account the results obtained during the entire period studied, we can establish that the 30 pollen/m³ threshold for moderate allergenic symptoms corresponded with 0.118 ng/m³ of alder allergen and 0.280 ng/m³ of Bet v1 protein in the atmosphere, and the 50 pollen/m³ threshold of high allergenic incidence with 0.138 ng/m³ and 0.286 ng/m³ respectively. An average of 18 days/year with high potential allergy risk due to *Alnus* pollen was registered along the study period, with the highest value observed in 2018 with 23 days. Additionally, a high sensitization potential risk caused by the *Alnus* allergen concentrations was recorded on an average of 14 days/year, with the highest value of 16 days observed in 2019 (Table 2, Fig. 2). In the case of *Betula*, an average of 16 days/year with high potential risk of allergy due to pollen and 22 days/year to allergen were registered, with the highest values observed in 2018 with 18 days for pollen and 16 days for the Bet v 1 protein (Table 2, Fig. 2).

In order to know the individual influence of the main meteorological variables in the airborne pollen and aeroallergen content of the atmosphere, a Spearman's correlation test was applied for each year separately and for the 2016–2019 data set. Significant positive correlations ($p < 0.01$) were observed between *Alnus* pollen and its allergen atmospheric content, and with maximum temperature. The strongest correlation was recorded for pollen vs allergen ($R^2 = 0.874$, $p < 0.01$) in 2016.

Table 1

Alnus and *Betula* start, end and length of the main pollen season (MPS). Pollen during the main pollen season, total pollen (pollen), pollen peak (pollen/m³), date of the pollen peak (day), and allergens during the pollen season, total allergen (ng), allergen peak (ng/m³), date of the allergen peak (day), finally (AP) pollen allergen potency (ng/pollen).

	<i>Alnus</i>					<i>Betula</i>				
	2016	2017	2018	2019	Average	2016	2017	2018	2019	Average
MPS start (day)	6-Jan	6-Jan	13-Jan	11-Jan	9-Jan	20-Apr	27-Mar	13-Apr	14-Mar	3-Apr
MPS end (day)	14-Feb	27-Feb	21-Feb	24-Feb	21-Feb	11-May	23-Apr	8-May	8-May	5-May
MPS length (days)	39	53	40	44	44	22	28	26	56	33
Pollen (pollen)	5036	2730	6152	2097	4004	7781	3024	8312	843	4990
Peak (pollen/m ³)	617	408	867	337	557	1255	307	1283	93	735
Peak date	24-Jan	30-Jan	24-Jan	25-Jan	25-Jan	3-May	10-Apr	25-Apr	31-Mar	17-Apr
	Allergen					Allergen Bet v1				
	2016	2017	2018	2019	Average	2016	2017	2018	2019	Average
Allergen (ng)	7.186	9.085	6.845	6.678	7.448	5.627	9.107	8.214	18.836	10.446
Peak (ng/m ³)	0.759	1.868	1.320	1.438	1.347	0.552	0.925	0.624	1.460	0.890
Peak date	27-Jan	1-Feb	18-Feb	27-Jan	2-Feb	19-Apr	19-Apr	6-May	31-Mar	18-Apr
AP (ng/pollen)	0.001	0.003	0.001	0.003	0.002	0.001	0.003	0.001	0.022	0.007

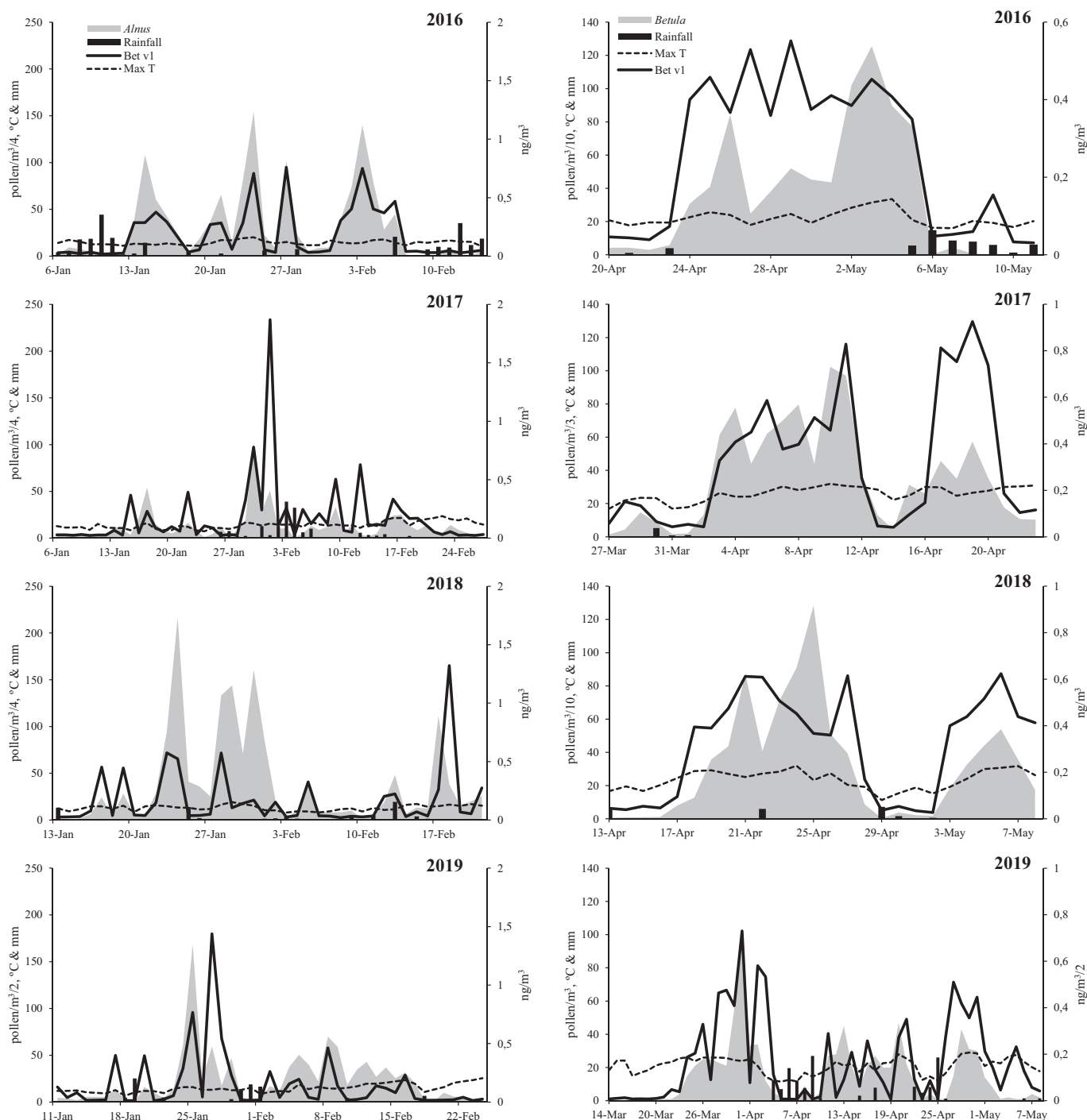


Fig. 1. *Alnus* and *Betula* pollen concentration (grey area), aeroallergen Bet v1 concentration (dark line) maximum temperature (dashed line and rainfall (bars) during the study years).

Table 2

Alnus and *Betula* pollen and allergen thresholds and risk days during the study years considered individually and both together.

		Pollen					Allergen					Pollen + Allergen	
		Risk (days)		Thresholds (pollen/m ³)			Risk (days)		Thresholds (ng/m ³)			Risk (days)	
		Moderate	High	Low	Moderate	High	Moderate	High	Low	Moderate	High	Moderate	High
<i>Alnus</i>	2016	4	19	1–30	31–50	>50	0	16	0.001–0.118	0.118–0.138	>0.138	4	19
	2017	13	17				3	16				15	23
	2018	6	23				0	15				6	23
	2019	8	14				3	13				11	18
<i>Betula</i>	2016	3	13	1–30	31–50	>50	0	12	0.001–0.280	0.281–0.286	>0.286	3	13
	2017	5	17				1	14				5	17
	2018	1	18				0	16				1	20
	2019	6	2				1	22				7	22

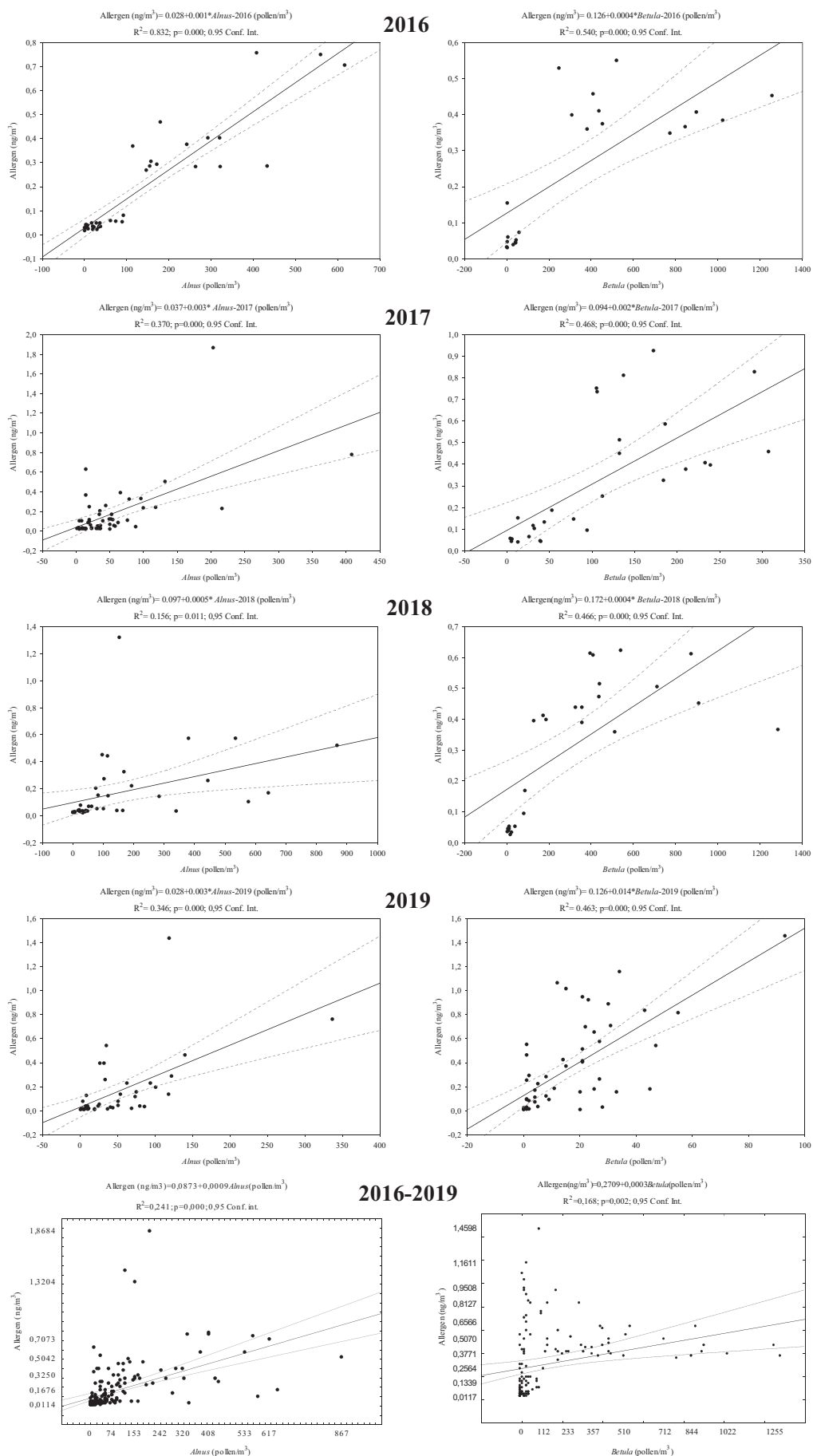


Fig. 2. Pollen concentrations (pollen/m³) versus allergen values (ng/m³) during the Main Pollen Season for each taxa, yearly and for the entire studied period (2016–2019).

Table 3

Spearman correlations between *Alnus* and *Betula* pollen or allergen and the main meteorological variables during MPS in each year and in the period 2016–2019, signification level ($p < 0.01^{**}$, $p < 0.05^{*}$).

	<i>Alnus</i>					<i>Betula</i>				
	2016	2017	2018	2019	2016–2019	2016	2017	2018	2019	2016–2019
Pollen										
Allergen	0.874**	0.542**	0.751**	0.689**	0.715**	0.754**	0.795**	0.802**	0.708**	0.603**
Average Temperature	−0.130	0.227	0.255	0.250	0.130	0.481*	0.433*	0.717**	0.490**	0.611**
Maximum temperature	0.281	0.378**	0.452**	0.358*	0.324**	0.764**	0.548**	0.739**	0.489**	0.604**
Minimum temperature	−0.278	0.050	−0.029	0.101	0.001	−0.533*	−0.047	0.581**	0.180	0.228**
Dew point	−0.253	0.064	−0.026	0.112	0.005	−0.609**	−0.011	0.536**	0.147	0.199*
Relative humidity	−0.340*	−0.397**	−0.463**	−0.317*	−0.318**	−0.839**	−0.467*	−0.409*	−0.361**	−0.470**
Rainfall	−0.479**	0.020	−0.108	−0.257	−0.172*	−0.638**	−0.460*	−0.518**	−0.369**	−0.435**
Wind speed	−0.389*	0.162	0.153	0.108	0.019	−0.144	−0.352	−0.205	−0.095	−0.078
Allergen										
Pollen	0.874**	0.542**	0.751**	0.689**	0.715**	0.754**	0.795**	0.802**	0.708**	0.603**
Average temperature	−0.157	0.168	0.330*	0.068	0.090	0.188	0.520**	0.734**	0.503**	0.539**
Maximum temperature	0.259	0.209	0.464**	0.190	0.231**	0.637**	0.457*	0.722**	0.523**	0.572**
Minimum temperature	−0.278	0.135	0.050	−0.044	0.000	−0.627**	0.232	0.559**	0.065	0.104
Dew point	−0.319*	0.124	0.052	−0.078	−0.016	−0.655**	0.046	0.524**	0.029	0.066
Relative humidity	−0.434**	−0.220	−0.445**	−0.219	−0.280**	−0.718**	−0.469*	−0.441*	−0.465**	−0.509**
Rainfall	−0.490**	0.262	−0.351*	−0.256	−0.153*	−0.620**	−0.409*	−0.415*	−0.421**	−0.452**
Wind speed	−0.317*	0.262	0.065	0.130	0.074	−0.228	−0.160	−0.348	−0.110	−0.137

Significant negative correlations ($p < 0.01$) were also observed between *Alnus* airborne pollen or its allergen concentrations and the relative humidity and rainfall variables. The highest negative correlation was registered between *Alnus* allergen and rainfall ($R^2 = -0.490$, $p < 0.01$) in 2016 (Table 3). In addition, significant positive correlations ($p < 0.01$) were observed between *Betula* pollen or the Bet v1 atmospheric content and the maximum and average temperatures. The strongest correlation was recorded for pollen vs allergen ($R^2 = 0.802$, $p > 0.01$) in 2018. Significant negative correlations ($p < 0.01$) between *Alnus* airborne pollen or its allergen concentrations and the dew point, relative humidity and rainfall were observed. The highest negative correlation was registered between *Betula* pollen vs relative humidity in 2016 (Table 3).

Finally, for the purpose of ascertain the altogether weather variables influence on airborne pollen and aeroallergen concentrations, a Principal Component Analysis was conducted. The objective of this analysis was to obtain a small number of linear combinations of the nine variables that account for most of the data variability. In the case of *Alnus* four components were extracted (with eigenvalues greater than or equal to 1.0) accounting for the 86% of variability (Table 4). This data suggests that four hypothetical (background) variables explained the

majority of total variance with a 14% loss of information. The four PCs were correlated as follows: Component 1 average and minimum Temperatures and Dew point, Component 2 maximum Temperature and Relative humidity, Component 3 Rainfall and Wind speed, and Component 4 pollen and allergen. The PCA analysis conducted for *Betula* extracted three components with eigenvalues greater than or equal to 1.0, accounting for the 76% of the variability of the original data (Table 4). The three hypothetical (background) variables explained the majority of total variance with a 24% loss of information. The three PCs were correlated as follows: Component 1 average and maximum Temperatures, Relative humidity and Rainfall, Component 2 minimum Temperature and Dew point, and Component 3 Wind speed. The results of both *Alnus* and *Betula* PCs analysis were plotted on two-dimensional graphs (Fig. 3). The bidimensional factor loading analysis indicated that the nearest variables to the atmospheric allergen or pollen concentrations were the variables exerting the highest loading in the analysis. In general, the airborne pollen concentrations were influenced by the average Temperature for both taxa, whereas the allergen concentrations were influenced by the minimum Temperature in the case of *Alnus* allergens or the maximum Temperature for Bet v1 allergens. The pollen and allergen concentrations were high-related variables for both taxa.

Table 4

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

Components	<i>Alnus</i>				<i>Betula</i>		
	1	2	3	4	1	2	3
Eigenvalue	3.502	1.909	1.237	1.083	3.368	2.361	1.091
Percent of variance	38.916	21.211	13.742	12.029	37.420	26.235	12.118
Cumulative percentage	38.916	60.127	73.869	85.897	37.420	63.655	75.773
Allergen	0.014	−0.059	0.009	0.870	0.588	0.034	−0.222
Pollen	0.086	−0.093	−0.103	0.842	0.466	0.352	−0.111
Average temperature	0.937	−0.255	0.182	0.086	0.714	0.649	−0.051
Maximum temperature	0.574	−0.650	−0.294	0.043	0.872	0.207	−0.252
Minimum temperature	0.900	0.032	0.326	0.039	−0.003	0.925	0.199
Dew point	0.965	0.169	0.169	0.031	−0.132	0.958	−0.037
Relative humidity	0.105	0.948	−0.111	−0.147	−0.907	0.254	−0.122
Rainfall	0.223	0.259	0.752	−0.112	−0.736	0.174	−0.179
Wind speed	0.245	−0.345	0.827	−0.004	−0.073	0.108	0.956

4. Discussion

Birch and alder trees are the genera commonly implicated in the allergic rhinitis processes of sensitized people (Asam et al., 2015). The major innovation of our study was the first detection of the Aln g1 allergens in the atmosphere by using Bet v1 antibodies. This fact verified the cross-reactivity between the main allergens of the *Betula* and *Alnus* pollen. The cross-reactivity, in terms of immunology, refers to the ability of components of the immune system (for example, IgE antibodies and/or T cells) to recognize different antigens (Biedermann et al., 2019). Currently, the studies about cross-reactivity between aeroallergens and food allergens are limited by the lack of large and controlled cases (Popescu, 2015). Some authors reported data about sensitization (clinical or subclinical) to aeroallergens and a further development of cases of food allergy, where the percentage of sensitization detected in adults was around the 80% of the studied cases (Kütting and Brehler, 2001) and 25% in children (Cudowska et al., 2013).

Cross-reaction problems between the Betulaceae family can be exacerbated by a not accurate design of the urban ornamental vegetation or by the placement of cities crossed by big rivers with parks or gardens

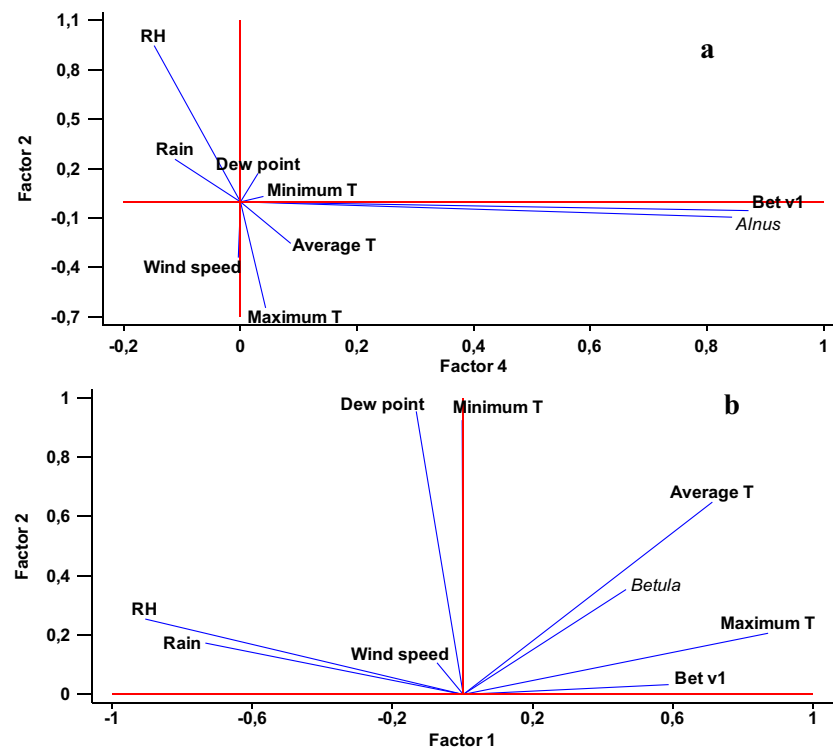


Fig. 3. Plot of Principal Components and Factor Analysis (PCFA) for each taxa (a—*Alnus* and b—*Betula*). Factor loadings Factor 4 vs. Factor 2 in *Alnus*, and Factor 1 vs. Factor 2 in *Betula*. Rotation varimax. Extraction Principal Components.

associated to its riverbanks, such as Ourense. *Alder* is a tree highly associated with the riverbank vegetation (Morales et al., 2011) with a MPS observed in the study area between January and February, as different authors pointed out for the Northwestern Spain (Jato et al., 2013; Rodríguez-Rajo et al., 2011a) or the North of Portugal (Ribeiro and Abreu, 2014). In addition, the *Betula* MPS occurred between April to May according with the data pointed out in Northwestern Spain (Jato et al., 2013; Rodríguez-Rajo et al., 2011a), several days earlier than in the North of Portugal (Ribeiro and Abreu, 2014). Only a short period of 4 weeks was observed between both flowering periods, reduced to only two in the case of the year 2019, where the end of the alder MPS occurred the February 24th and the start of the *Betula* pollen season the March 14th. The main consequence of these successive blooms in both trees was the so-called “priming effect”, which could hugely reduce the threshold concentrations for the symptoms appearance (Connell, 1969). The cross reactions detected in our study demonstrated that urban allergic population sensitized to *Betula* pollen could suffer allergic symptoms during winter (as a consequence of *Alnus* pollen allergens presence in the atmosphere), and later in spring can suffer the “priming effect” with the manifestation of higher symptoms under lower birch pollen grain levels in the atmosphere. This fact was observed by medical specialists in Allergology in the study area and was orally informed to our research group.

In addition, other influential factors should be taking into account in the urban environments to understand the high prevalence of pollen related cases of sensitized population regarding to the rural areas (D’Amato and Liccardi, 2002; Von Hertzen and Haahela, 2004; Nicolaou et al., 2005). The unsuitable green space construction by means of allergenic pollen species, pollution and urban heat island effect are considered the main causal agents (D’Amato and Liccardi, 2002; Von Hertzen and Haahela, 2004; Nicolaou et al., 2005). Temperature and pollution levels increase in the cities is associated with a higher expression of plant pathogenesis-related proteins (such as the *Aln g1* and the *Bet v1*) in the pollen from urban contaminated areas

(Rodríguez-Rajo et al., 2011b). Furthermore, fossil fuel combustions generate a particulate matter (PM) which could be adhered to the pollen grains surface acting as adjuvants to modify the microenvironment in which allergens are released from pollen, prompting an increase of the pollinosis effect (D’Amato and Liccardi, 2002; Le Souef, 2009; Traidl-Hoffmann et al., 2009).

Inter annual variations of the pollen and protein content in the atmosphere can be affected by weather variables, mainly temperature, relative humidity or rainfall (Buters et al., 2010). In the present study, a Spearman’s correlation analysis was conducted between pollen, aeroallergens and the main meteorological parameters in order to ascertain individual associations. Some variations were registered along the studied years due to the strong meteorological inter annual differences observed during the MPS. Considering the complete data set, the correlation analysis showed a positive Spearman’s correlation coefficient between maximum temperature and *Alnus* pollen or allergen concentration, and between maximum or average temperature and *Betula* pollen or allergen concentration. This analysis also showed a negative correlation coefficient between *Alnus* and *Betula* pollen or allergen and relative humidity or rainfall. These results accord with the pointed out for different taxa in the North of Portugal (Fernández-González et al., 2019) or Poland (Nowosad et al., 2015; Bilinska et al., 2019). Our study showed that the lowest total amount of *Betula* pollen and the highest amount of *Bet v1* allergen occurred in 2019. This fact can be linked to the high rainfall events registered in the middle of *Betula* MPS, since rainfall can act prompting atmospheric pollen washes but under strong rainfall conditions, some cytoplasmic material, such as proteins and starch granules, can be abruptly discharge from the pollen grains to the atmosphere (Fernández-González et al., 2011, 2013). We obtained similar results using the Principal Component Analysis (PCA), which identified the altogether influence of the meteorological parameters. The parameters that increased the *Alnus* and *Betula* distribution were maximum and average temperatures. On the contrary, rainfall and relative humidity reduced the *Alnus* and *Betula* pollen and

allergen concentrations. Similar findings using the PCA methodology have been reported by González-Parrado et al. (2009) for *Alnus*, García-Mozo et al. (2008) for *Olea* and Vara et al. (2016a) for *Fraxinus*. In addition, the PCA plot showed the strong relations between pollen and allergen concentrations in the air. Studies carried out across Europe noted that the own birch pollen grains are the main source of its allergens in the atmosphere as they are only detected in the same fraction of the cascade samplers than pollen grains (Buters et al., 2012).

In order to identify the days with risk of allergy produced by *Alnus* and *Betula* pollen and its aeroallergens, the pollen thresholds proposed by the Spanish Aerobiology Network were followed (three risk categories: Low 1–30 grains/m³, Moderate 31–50 grains/m³ and High >50 grains/m³). Furthermore, a regression equation between aeroallergen and pollen was developed in order to calculate the aeroallergen allergy risk threshold concentrations that corresponds with the marked pollen categories (Table 2). The combination of both variables helped us to know the real number of risk days that induce allergy in sensitized population, as in recent years different studies showed some discordances between the date of pollen and aeroallergen presence in the atmosphere (D'Amato et al., 2008; Rodríguez-Rajo et al., 2011b; Vara et al., 2016b; Fernández-González et al., 2019). In our study, the year 2019 registered 14 days with *Alnus* risk of allergy due to pollen concentrations and 13 days due to its aeroallergen concentrations. Nevertheless, only 9 of these days with high risk of allergies coincided considering both, airborne pollen and aeroallergens, which raised to 18 the real number of days with high risk of allergy. In the case of *Betula*, during the year 2018 there were registered the highest number of days with birch allergy risk, 18 days due to pollen concentrations and 16 days because of its aeroallergen concentrations. As in the case of alder, not all these days with high risk of allergies coincided considering both, airborne pollen and aeroallergens. This fact also elevated to 20 days the real number of days with high risk of allergy. Therefore, the traditional information to prevent allergies, such as pollen concentrations, should be combined with data of atmospheric allergen presence to identify the real allergenic load in the atmosphere and the precise number of days with allergy risk.

In general, the *Alnus* pollen peak took place before the allergen peak, and the aeroallergen peak was generally preceded by rainfall. This fact was previously pointed out by other authors (Moreno-Grau et al., 2006), as after soft rainfall episodes pollen grains deposited in different surfaces under wet conditions can suffer a hydration process that releases the allergenic proteins to the atmosphere.

5. Conclusions

The major innovation of our investigation was the first detection of the *Aln* g1 allergens in the atmosphere by using Bet v1 antibodies. The cross-reactivity between the pollen of the main genera of the Betulaceae family, used as ornamental flora in cities such as *Betula* or *Alnus*, was demonstrated. A high pollen allergen potency for *Alnus* and *Betula* in the years with the lowest airborne pollen concentration was detected, which may be directly related to meteorology, since in those years' rain storm episodes occur.

The main consequence of the successive bloom of both trees was the so-called "priming effect" that could hugely reduce the threshold concentrations for the symptoms appearance. Urban allergic population sensitized to *Betula* pollen could suffer allergic symptoms during winter (as a consequence of *Alnus* pollen allergens presence in the atmosphere), and later in spring with the manifestation of higher symptoms under lower birch pollen grains levels in the atmosphere.

To prevent allergies, data pollen concentrations should be combined with data of atmospheric allergen to identify the real allergenic load in the atmosphere in order to personalize antihistamine treatments in allergic patients.

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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