

***Betula* pollen: One of the most important aeroallergens in Ourense, Spain. Aerobiological studies from 1993 to 2000**

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Abstract

This study presents the results obtained for airborne *Betula* pollen between 1992 and 2000 in Ourense, Spain, sampled by volumetric spore-trap (LANZONI VPPS2000). Annual and year-on-year variations were analyzed, and a statistical study of the correlation between daily counts and several meteorological parameters was performed. Birch pollen is present in the atmosphere during March and April in Ourense. Significant differences were observed among the different years. Values obtained for the correlation coefficient between *Betula* pollen counts and the various meteorological parameters studied indicate, for Ourense, a positive correlation between pollen count and both temperature and sunlight. A negative correlation was recorded for relative humidity. Temperature is thus the determining factor for flowering onset and intensity. Regression equations included values for the days prior to pollen concentration measurement in order to optimize results.

1. Introduction

In Ourense, the *Betula* pollen type comprises only one native species, *Betula alba* L., Sp. Pl., although another species – *B. pendula* Roth, Tent. Fl. Germ – has been introduced. This arboreal species, widespread in Ourense, is closely associated with watercourses or damp grounds, although it can also invade stony areas and burned or deforested habitats. In recent years, *Betula pendula* has been planted as an ornamental in many gardens, since it is hardy, requires little care and grows quickly. Ourense lies at the boundary of the distribution area for this species, which makes *Betula* pollen behavior particularly worthy of study. Due to its location, certain weather phenomena parameters can lead to extreme situations for the species.

Betula pollen is the cause of symptoms of allergy in many people; D'Amato and Spieksma (1990) classified *Betula* as the main allergen in

Northern Europe, while Selle et al. (1992) reported patients with monospecific allergies in Italy. Elsewhere, Zawisza and Samolinska-Zawisza (1991) reported up to 10% of patients as being sensitive to *Betula*. Yli-Panula and Rantio-Lethimäki (1994) studied allergens belonging to this pollen type. Zawisza et al. (1993) examined specific IgE levels in a population during the flowering period. Ekebon et al. (1996) sought to identify the main allergen in the *Betula* pollen grain. Other researchers have addressed its morphology (Faber et al. (1996).

The aim of this work was to analyze the variations in *Betula* pollen concentrations and propose an statistical model for pollen variation.

2. Material and methods

Ourense is situated in north-western Spain. Its climate is oceanic with a strong Mediterranean

influence. Mean annual temperature is 14 °C (maximum average 18.9 °C, minimum average 9.2 °C). Annual rainfall is 772 mm, with a highly irregular distribution throughout the year; summer rainfall is only 29 mm (Carballeira et al., 1983).

The city is located at 130 m above sea level, in a depression caused by the rivers Loña, Barbaña and Miño (Nespereira, 1982). The area is surrounded by steep-sloped mountains of up to 400 m above sea level. The geographical coordinates are 42.0° N and 7°51' W. From a biogeographical standpoint, Ourense lies at the confluence of the Eurosiberian and Mediterranean regions (Izco, 1988; Peinado-Lorca and Rivas-Martinez, 1988). The dominant vegetation comprises oaks, mainly *Quercus robur* intermingled with *Q. pyrenaica* and *Q. suber* in lower-lying areas, and *Betula alba* in higher areas. Due to both forest fires and neglect, wooded areas have decreased considerably and trees have been replaced by bushes (*Cytisus*, *Ulex*, *Adenocarpus*, Ericaceae, etc.). *Alnus glutinosa*, various *Salix* species and *Fraxinus angustifolia* are found along the rivers. The main crops are potatoes, cereals, corn, barley, turnips and grapes.

A Hirst-type spore trap (Lanzoni VPPS 2000) operated throughout the study period; the trap was placed at about 25 m above ground level on the roof of the Cristal-Piñor Hospital, located in a semi-urban area of the city. From 1 January 1993 to 31 December 2000, *Betula* pollen was monitored uninterruptedly.

The methodology used for sampling and processing aerobiological data was that proposed by the Spanish Aerobiology Network (R.E.A.) (Domínguez et al., 1991). The adhesive substance used was 2% silicon solution in carbon tetrachloride. Samples were slide-mounted in fuchsin-stained glycerol gelatin and four longitudinal strips were examined at 400× magnification through a standard light microscope in order to obtain daily counts. Counts were taken every hour and mean daily values were expressed as number of pollen grains per cubic meter of air. The limits of the main pollination period were set at 95% of the whole annual pollen-production period (Torben, 1991). Meteorological data were obtained from the Local Area Meteorological Service of Ourense, just 20 m from the sampling point. The STATISTICA V4.5 statistical package was used for statistical data processing. In order to

determine the influence of meteorological parameters on pollen concentration, a regression analysis was performed using the log normal transformation of pollen count values as independent variable, while the meteorological parameters displaying the best correlation were used as dependent variables. In order to diminish the statistical variability of initial regression analysis data, a Scheffé test was performed for all study years; any year displaying significantly deviant values was excluded from the regression analysis.

The regression equations thus obtained were tested during the final year of the study in order to determine their value as pollen-concentration predictors.

Spieksma et al. (1995) has highlighted the influence of previous temperatures on the start of flowering. In order to determine this influence, prior temperatures were examined. Heat accumulation starts when temperatures rise after the winter period, and stabilize at over 10 °C.

Several tests were carried out using different accumulation base temperatures. Heat accumulation values starting from 10 °C were used, since this was the best temperature for achieving least variability in the data for the different years. In winter, the number of days with sub-zero temperatures occurring before heat accumulation was counted.

3. Results

3.1. Interannual variations

Betula pollen appears in the Ourense atmosphere from the last ten days in March until the beginning of May. The highest values were recorded from late March until mid-April. In 1994, peak values were not recorded until late April, although there was a previous period of high values coinciding with the usual period of maximum annual values.

The highest daily values ranged from 37 pollen grains m⁻³ (in 1998) and 254 pollen grains m⁻³ (in 1994). Peaks recorded in 1998 were much lower than those of other years (Figure 1). Similar variations were also observed for total annual pollen counts. El-Ghazaly et al. (1993) link these variations to flowering cycles; the period studied here was not long enough to enable confirmation of this.

With regard to maximum and percentage values, *Betula* accounts for a considerable

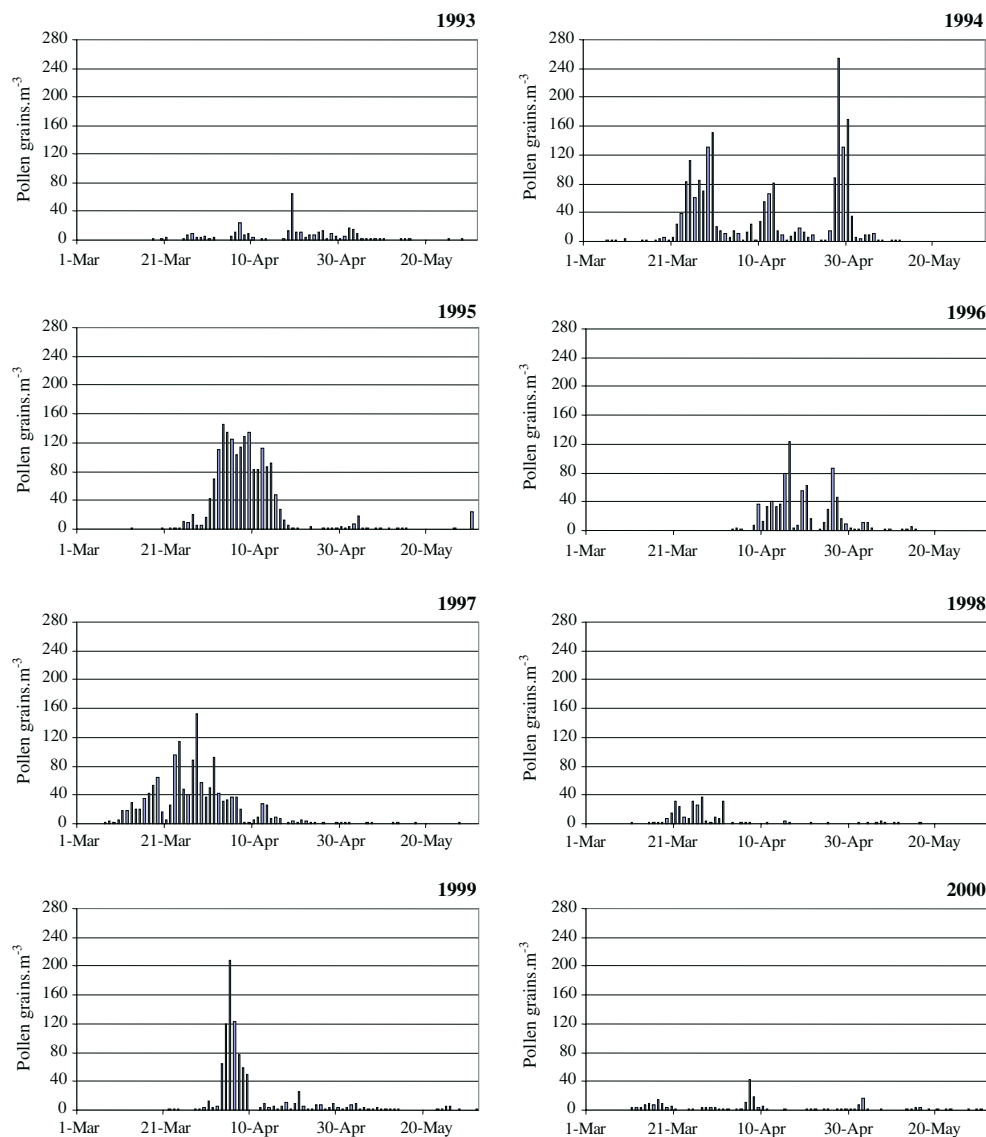


Figure 1. Pollen count trends over the sampling period: dates (x axis) plotted against airborne *Betula* pollen counts (y axis). Values are expressed in pollen grains m^{-3} .

percentage of total annual pollen, despite fluctuations over the sampling period. *Betula* represented as much as 8% of the annual pollen count in 1994 and 1995, and as little as 1.4% in 1993 and 1.5% in 1998; in other years, it accounted for an average of 5% of total pollen (Table 1).

The mean annual count was 1003 pollen grains, although there were considerable year-on-year variations in both total annual *Betula* pollen counts and in *Betula* as a percentage of total annual pollen. These variations may have been

prompted either by natural variations in flowering or by the environmental conditions in which flowering took place; lower values were related to higher rainfall.

There were also considerable variations in the duration of the main pollination period (mean 46 ± 28 days), which was longer in years when fewer pollen grains were collected, i.e., 1993, 1998 and 2000 (duration >50 days). These years recorded the highest rainfall and the lowest temperatures of the study period.

Table 1. Principal values for *Betula* periods during the studied periods. Mean temperature is expressed in °C and the rainfall in mm

	1993	1994	1995	1996	1997	1998	1999	2000
Σ (gp/m ³)	354	1978	1839	813	1465	244	856	472
Annual percentage	1.4	8.4	8.5	3.9	6.7	1.5	6.5	2.7
M.P.P.	24-3/20-5	21-3/2-5	27-3/8-5	9-4/8-5	12-3/15-4	19-3/7-5	1-4/9-5	12-3/20-5
Duration	58	43	43	30	35	50	39	70
Maximum Value	66	254	146	123	152	37	209	43
Date	19-4	28-4	3-4	16-4	28-3	27-3	5/4	7/4
Mean T ^a MPP	12.9	13.4	15.3	14.	15.8	14.6	14	13.2
Rainfall. MPP	204	43	56	47	14.5	224.9	142.6	243

The highest daily counts were recorded when high temperature and low relative humidity (about 60%) were observed; pollen counts fell considerably on rainy days.

3.2. Statistical analyses

In order to check the results obtained, values for 2000 were excluded from statistical analysis. Before computing any statistical analysis, the normal distribution of the data set was tested. The results revealed abnormal distributions in a large number of cases. In order to perform the different analyses, log normal pollen count values were calculated and a unit was added to the original value (i.e. $\ln(x + 1)$) in order to avoid the problems caused by null values. The modified variable was then tested using the Kruskal–Wallis test and adjusted to a normal distribution.

Variance analysis yielded an *F* value of 20.14, associated with a probability value of <0.001, which implied the existence of significant differences between study years. A Scheffé test was performed in order to determine these differences; the results are shown in Table 2. Significant differences were observed between different studied years. These differences may be ascribed to

weather-related factors: in 1993 and 1998, low temperatures and higher rainfall were recorded during the main pollination period. The Scheffé test showed similar values for these parameters and for pollen counts for the two years concerned.

3.3. Correlation with meteorological parameters

In order to study the correlation between *Betula* pollen counts and weather-related parameters, we considered both the main pollination period as a whole and the days prior to the peak pollen count.

Overall analysis excluded 1993 and 1998, shown by the Scheffé test to be statistically different. Mean temperature was not significant. The maximum temperature is significant when years are analyzed individually. Therefore, *Betula* displayed a stable positive correlation with maximum temperature; however, the correlation with minimum temperatures was not significant, except in the last two studied years, as shown in Table 3.

A significant negative correlation was observed for relative humidity over the study period as a whole, and also for three of the six study years; a similarly significant negative correlation was observed for rainfall in four of the study years.

Table 2. Scheffé test values for individual study years

	1993	1994	1995	1996	1997	1998	1999
1993							
1994	0.000001*						
1995	0.003395*	0.815993					
1996	0.013767*	0.888553	1.000000				
1997	0.000000*	0.987014	0.345754	0.481719			
1998	0.763829	0.000000*	0.000005*	0.000072*	0.000000*		
1999	0.530586	0.043792*	0.700494	0.771045	0.004374*	0.024396*	

Table 3. Values for Pearson's correlation coefficient between the pollen values and the different meteorological parameters. Values with $p < 0.05$ are marked (*) and values with $p < 0.01$ are marked (**). Values for LnBETU1, ...LnBETU7, corresponding to log-normal pollen counts for 1, 2, 3, ... up to 7 days before

	1993	1994	1995	1996	1997	1998	1999
Rainfall	-0.2114	-0.4237**	-0.4230**	-0.5230**	-0.4888**	-0.1707	-0.2651
H%	-0.4388**	-0.2735	-0.7780	-0.5103**	-0.3018	0.3862**	-0.2714
MAX T ^a	0.4294**	0.6775**	0.5842	0.6077**	0.0728	-0.5534**	0.5051**
MEAN T ^a	0.2807*	0.5909**	0.3575*	0.4815**	-0.4056*	-0.5734**	0.4775**
MIN T ^a	-0.0262	0.1889	-0.0878	-0.0147	-0.5775**	-0.5061**	0.2389
INSOLATION	0.2333	0.5248**	0.6961**	0.3632*	0.6927**	0.3174*	0.3458*
LnBETU1	0.6360**	0.6714**	0.9240**	0.6028**	0.7015**	0.7263**	0.6349**
LnBETU2	0.3414**	0.3724*	0.8474**	0.0738	0.2685	0.6291**	0.3081
LnBETU3	0.0905	0.1190	0.7610**	-0.1043	0.1557	0.4930**	0.2505
LnBETU4	-0.0536	-0.1977	0.6966**	-0.0124	0.2395	0.5548**	0.1806
LnBETU5	-0.0063	-0.2396	0.6194**	0.0744	0.2946	0.6087	-0.0121
LnBETU6	0.0055	-0.2294	0.5290**	-0.0023	0.1896	0.4514**	-0.2219
LnBETU7	-0.0347	-0.2263	0.4337	-0.0629	0.0087	0.2709	-0.3198

Both sunlight and temperature displayed a significant positive correlation with pollen counts over the study as a whole and for the three of the individual study years.

Correlation coefficient values remained constant over the study as a whole; generally speaking, the correlation was greater when values over pre-peak days were taken into account. During this period, there is less drying-up of flowers, and phenological status is more uniform, thus avoiding factors that might mask weather-related effects.

With regard to temperature, attention is drawn to the considerable reduction in correlation coefficient values for the study as a whole, in comparison to individual years; this is especially true of average temperature, which did not record significant values for the study as a whole, despite attaining high values for individual years.

The correlation with pollen counts on previous days was also analyzed, and provided a wealth of information regarding the flowering period that was not obtained from weather-related parameters. A highly-significant correlation was recorded for *Betula* pollen concentrations measured on the previous day; thereafter, however, significance decreased sharply.

3.4. Regression analysis

Regression analysis was performed with maximum temperature and log-normal pollen count on

the previous day as independent variables, and log-normal pollen count as the dependent variable, in order to account for pollen behavior during the main pollination period. A first-order polynomial expression was obtained. ANOVA analysis showed that the model was significant in all study years, accounting in some cases (e.g. 1995) for up to 85% of the information; this percentage was lower in other years, though remaining over 50%, with the exception of 1993, 1996 and 1999 when it fell to 42, 43 and 45% respectively. Values and equations are shown in Table 4.

Regression analysis revealed a clear correlation with temperature. However, the straight line coefficients for the different parameters varied between all the years studied in terms of both temperature and pollen concentration on the previous day. Temperature and correlation coefficients were negative in 1997, 1998 and 1999.

3.5. The onset of the main pollination period

Analysis of temperature oscillations in the period prior to flowering showed that flowering started when temperature increased and remained at constant average high values, i.e., over 10 °C. A cold period, even with sub-zero temperatures, was necessary prior to flowering. The duration of the cold period varied considerably; in 1993, sub-zero temperatures were recorded on 24 days,

Table 4. Regression summary and equations obtained for individual study years. F value (Snedecor's F distribution), p value (probability value for F), r^2 value (square r). The total equation excludes the years 1993 and 1998

Year	Equation	F	p	R^2
1993	$\text{Ln Betu} = -0.0923 + 0.0514 (\text{t avg}) + 0.5976 (\text{ln Betu1})$	20.309	0.0000	0.425
1994	$\text{Ln Betu} = 0.0455 + 0.1141 (\text{t avg}) + 0.4870 (\text{ln Betu1})$	21.621	0.0000	0.519
1995	$\text{Ln Betu} = -0.1678 + 0.0224 (\text{t avg}) + 0.9177 (\text{ln Betu1})$	119.56	0.0000	0.856
1996	$\text{Ln Betu} = -1.5181 + 0.194 (\text{t avg}) + 0.4982 (\text{ln Betu1})$	10.547	0.0004	0.438
1997	$\text{Ln Betu} = 2.2452 - 0.0712 (\text{t avg}) + 0.6603 (\text{ln Betu1})$	16.148	0.0000	0.502
1998	$\text{Ln Betu} = 1.5513 - 0.0791 (\text{t avg}) + 0.5868 (\text{ln Betu1})$	33.717	0.0000	0.589
1999	$\text{Ln Betu} = -0.5764 + 0.1036 (\text{t avg}) + 0.5554 (\text{ln Betu1})$	15.285	0.0000	0.459
Total	$\text{Ln Betu} = 0.0786 + 0.0198 (\text{t avg}) + 0.8107 (\text{ln Betu1})$	377.46	0.0000	0.674

whereas in other years the cold period was substantially shorter. During the period prior to the 1998 main pollination period, temperatures remained high, not dropping below zero.

After this cold period, the trees started to accumulate heat until flowering took place; this coincided with the moment when temperatures increased and accumulated heat was about 270 °C, although this value was also variable (see Table 5): in 1996 and 1998, for example, the previous winter was mild, and temperatures never fell below zero. Absence of a preceding cold spell hindered flowering, which when it took place was not very intense. This was particularly significant in 1998, when the total pollen count was 244 grains.

3.6. Predicting the onset of the main pollination period

To test the reliability of this equation as a pollen predictor, the estimates obtained were compared with real values for 2000 (Figure 2). The two series displayed similar trends, despite slight differences with regard to maximum values. A χ^2 analysis to test for differences yielded the value $\chi^2 = 377.4693$,

i.e. for 69 degrees of freedom, the probability was close to 0 (Table 6).

4. Discussion

4.1. Year-on-year variation

The main *Betula* pollination period for Ourense largely coincides with those reported for other towns in Galicia, although the values are very different: higher values have been recorded in Atlantic cities such as Santiago de Compostela (Aira et al., 1998), except in 1996, and lower values are reported for Vigo (Rodríguez et al., 1998).

Betula pollen accounted for a smaller proportion of the total pollen count than in Northern European countries, where the birch is more common. Indeed, D'Amato and Spieksma (1990) cite *Betula* pollen as the single most abundant in Northern and Western Europe, while Johansen (1991) report that it accounts for 27% of total annual pollen in Norway. Here, in contrast, it accounted for no more than 8.5% of total annual pollen, and in some years (e.g. 1998) did not even

Table 5. Values for the cold period and the accumulated temperature period. The values given for cold period is the number of days

	1993	1994	1995	1996	1997	1998	1999
Cold period	24	13	14	17	10	0	23
Accumulated from	3/3	20/2	10/3	19/3	20/2	34	8/3
Days accumulated	15	10	16	22	20	34	19
Accumulated hot	248	258	294	446	273	478	261
MPP start	24/3	21/3	27/3	10/4	12/3	19/3	1/4

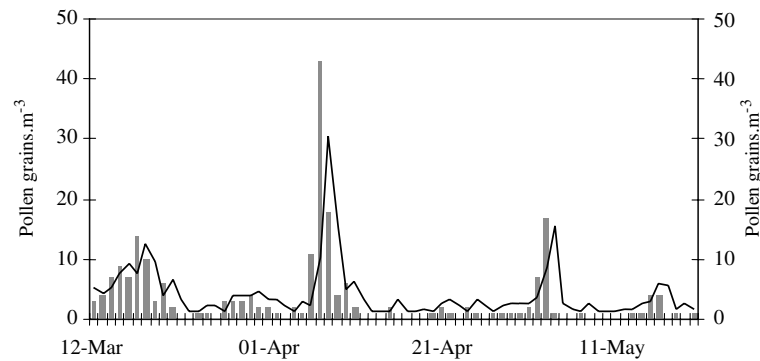


Figure 2. Observed and predicted values for *Betula* pollen using the obtained total equation for the main pollination period in 2000. The bars represent the observed values and the line the predicted values.

exceed 1%. Goldberg et al. (1988) also report considerable variations from year to year.

Significant variations were observed in the length of the main pollination period; similar variations in duration have been recorded for previous years in Ourense (Iglesias, 1990), in Santiago de Compostela (Aira et al. 1998), and in various parts of France (Ruffaldi & Greiffier, 1991). Johansen (1991) also observed considerable variations duration of the main birch pollination period in Norway. Significant variations have also been reported with regard to pollen distribution over the pollination period (Atkinson & Larson, 1990).

Since *Betula* species are widely distributed over the study area, the influence of long distance transport may vary from year to year; Johansen (1991) and Hjelmroos (1991) studied long distance transport and reported the presence of birch pollen in places where significant flowering had not yet taken place – indeed, amounts may be sufficient to cause symptoms in birch-sensitive allergy-sufferers (Wallin et al., 1991). The effect of airborne pollen transport in Ourense has been highlighted by Iglesias et al. (1988) who note that there are few birches in the vicinity of the sampling point. Variation in weather conditions during the flowering period may also affect its duration; flowering lasted longest in the coldest years, and an average temperature of 14–16 °C during the main polli-

nation period was found for the years with the shortest flowering periods, contrasting with only 13 °C in 1993. This is one reason why *Betula* displays a longer flowering period in Northern areas (Jäger et al., 1996). In León (Spain), Fernández González et al. (1998) observed shifts in the pollen curve of up to two weeks due to weather conditions.

4.2. Statistical analysis

Temperature has a decisive effect on airborne *Betula* pollen concentrations (Ramfjord, 1991), although temperature patterns are in turn considerably influenced by other weather-related parameters such as rain or relative humidity; correlation with these parameters was highlighted by Atkinson & Larson (1990). Here, temperature displayed a positive significant correlation with pollen counts over the study period. In nearby cities such as Santiago de Compostela (Aira et al., 1998b), *Betula* displays positive correlation with maximum and minimum temperatures, and negative with rainfall. In contrast, the coefficient of correlation with minimum temperatures was negative in Ourense in many years.

Analysis of the scatter chart obtained by regression analysis shows a different distribution pattern each year. Regression analysis for the study period as a whole yielded a more circular scatter chart, with a random distribution of points. Correlation coefficient values were therefore considerably reduced.

Although the correlation between pollen counts and temperature remained constant, temperatures always exerting a positive influence on airborne

Table 6. Values for the χ^2 analysis between observed values for pollen concentrations during 2000 and predicted values for the same period using the equation for total years

χ^2	Degree free	<i>p</i>
377.4693	69	0.0000

pollen concentrations, numerical ratios differed considerably from year to year. For the last two study years the slope of regression was inverted due to outlier values; on many days over this period pollen counts were either very low or non-existent.

In order to obtain an equation providing sufficient information to chart *Betula* pollen evolution over the pollination period, pollen counts for the previous day were included, which yielded more satisfactory values for the regression equation. The regression equation developed using the values recorded over the study period (Table 4), served as a model for predicting pollen concentrations.

Experimentally-obtained values for mean daily pollen counts in 2000 were similar to those predicted by the regression equation, although recorded one day earlier.

The data obtained from the regression equation displayed the same trends as experimentally-observed values, as confirmed by χ^2 analysis (Table 6). Thus, the equation is valid for predicting *Betula* pollen concentrations in Ourense.

4.3. Predicting the start of the main pollination period

Heat accumulation values for 1993 and 1998 were similar, reaching an average 267 °C; similar values were observed in Ruffaldi and Greiffier (1991), who reported an accumulated temperature value of approximately 280 °C at the start of pollination.

During the year 2000, accumulated temperature values rose to 409 °C, and temperatures below 0 °C were recorded on 26 days. During the heat accumulation period, sub-zero temperatures were also recorded, which may account for the high values for the year.

In the present study, values for predicting flowering onset varied from year to year, particularly with regard to the length the required cold period. Any change in the length or intensity of the cold period prompts alterations in the onset and intensity of flowering; this occurred 1998, for example, when the weather was unseasonably mild. This dormancy model, governed mostly by temperature, may also be affected by other parameters; some authors have drawn attention to the influence of photoperiod on *Betula* flowering (Andersen, 1991).

The influence of the previous year on *Betula* flowering has been highlighted by Emberlin et al. (1990) for pollen in London. These authors also noted phenological rhythms in the flowering of certain taxa. Andersen (1991) proposes a prediction model that takes into account not only the cold affecting the plant during winter but also the heat affecting it at a later stage. Emberlin et al. (1997) also took temperature into account when studying the possible effects of climate change, particularly temperature increases, on the timing of *Betula* flowering.

5. Conclusions

Betula pollen remains in the atmosphere for three months – from March to May – and accounts for an average 5% of total annual pollen.

The values recorded for total annual *Betula* pollen were extremely variable, ranging from 1.978 grains in 1994 to 244 in 1998. Total annual counts were influenced by weather conditions; the lowest counts were recorded in years with higher rainfall and lower temperatures.

Maximum daily annual values – usually recorded by mid-April – also fluctuated considerably, varying between 37 and 254 grains of pollen/m⁻³ in 1998 and 1994, respectively.

Temperature seems to be the key factor governing the timing and intensity of *Betula* flowering, determining both the onset of pollination and the amount of airborne pollen. However, the relationship between the two parameters is highly variable, and pollen behavior cannot be predicted solely on the basis of temperature patterns.

Pollen counts on the previous day are useful for modeling and accounting for predicted airborne *Betula* pollen concentrations, because this parameter is in itself a compilation of all the factors influencing flowering.

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