

## EFFECT OF AIR TEMPERATURE ON FORECASTING THE START OF CUPRESSACEAE POLLEN TYPE IN PONFERRADA (LEÓN, SPAIN)

Carmen Reyes Fuertes-Rodríguez, Zulima González-Parrado, Ana María Vega-Maray, Rosa María Valencia-Barrera, Delia Fernández-González

Área de Botánica, Universidad de León, León, Spain

Fuertes-Rodríguez CR, González-Parrado Z, Vega-Maray AM, Valencia-Barrera RM, Fernández-González D: Effect of air temperature on forecasting the start of Cupressaceae pollen type in Ponferrada (León, Spain). *Ann Agric Environ Med* 2007, **14**, 237-242.

**Abstract:** In order to survive periods of adverse cold climatic conditions, plant requirements are satisfied by means of physiological adaptations to prevent cells from freezing. Thus, the growth of woody plants in temperate regions slows down and they enter into a physiological state called dormancy. In order to identify the chilling and heat requirements to overcome the dormancy period of Cupressaceae pollen type in the south of Europe, we have carried out our study with aerobiological data from a 10-year (1996-2005) period in Ponferrada, León (Spain). For the chilling requirements the best result was with a threshold temperature of 7.1°C and an average of 927 CH. Calculation of heat requirements was carried out with maximum temperature, with 490 growth degree days (GDD) needed, with a threshold temperature of 0°C. We have used the 2002-2003, 2003-2004 and 2004-2005 periods in order to determine the real validity of the model. We have not used these years in developing the models. The dates predicted differ in only a few days from those observed: in 2002-2003 there was a difference of 11 days, in 2003-2004 predicted and observed dates were the same, but in 2004-2005 the difference obtained was of 43 days.

**Address for correspondence:** Carmen Reyes Fuertes-Rodríguez, Departamento de Biodiversidad y Gestión Ambiental (Botánica), Facultad de Ciencias Biológicas y Ambientales, Universidad de León. Campus de Vegazana, 24071 León, Spain.  
E-mail: crfuer@unileon.es

**Key words:** forecast, dormancy, Cupressaceae, pollen, Ponferrada, aerobiology, chilling requirement, heat requirement.

### INTRODUCTION

The physiological behaviour of woody plants with winter-spring flowering is subject to constant variations due to the action of different bioclimatic parameters. Before flowering, temperature plays a particularly important role in the maturation of reproductive organs and is the primary factor influencing the growth and development of plants and pollen production. During the flowering period, secondary factors, such as sunshine, rainfall and relative humidity, determine the opening of anthers and the release of pollen grains. Airborne pollen is greatly influenced by

meteorological conditions and is dispersed by a tertiary factor, the wind, which results in the scattering of pollen in the atmosphere [20].

Nowadays, a number of different indices have been referenced in order to determinate chilling and heat requirements. The period of rest is usually determined by the number of chilling units or chilling hours [3]. The heat requirements for overcoming the dormancy period are frequently measured as degree-hours, where 1 degree-hour is equivalent to 1 hour when 1°C above a lower threshold temperature.

Different models predicting the dates of budburst or flowering have been described, some of which consider

only the heat requirements [5], and others also consider the action of chilling [16, 19, 23]. Phenological data have usually been used to calculate chilling and heat requirements, and to predict the onset of different phenological phases [36, 37, 40]. On the assumption that atmospheric pollen indicates the time of pollination, aeropalynological data are also employed to determine the onset of the pollen season [10, 11, 12, 14, 18, 33]. In all cases, the aim is to find predictive models that enable us to ascertain the onset and severity of the pollen season. This has a particular importance for people allergic to the Cupressaceae pollen type, who can then start anti-allergic treatment several days before the beginning of pollination, thereby optimising its effectiveness.

Cupressaceae pollen type is well known as the first cause of invernall allergy in Europe [6]. Certain species of this botanic family, such as *Cupressus sempervirens* and *C. arizonica*, can produce considerable amounts of pollen between 5-10 years after they were planted. Moreover, in other species of Cupressaceae, certain parasitic species of fungus may induce an increase in their pollen production [2, 28]. All of these could cause an increase of Cupressaceae hay-fever cases together with air pollution. Panzani *et al.* [26] suggested an adjacent action of the aluminium silicate found in air pollution. Other researches [25] involve diesel engine particles in these allergy cases. But in recent studies with Cup a 3, a thaumatin-like protein (TLP) of *Cupressus arizonica* pollen grains, Suárez-Cervera *et al.* [39] pointed out that Cup a 3 observed is higher in pollen from *C. arizonica* growing in industrial areas than in pollen collected from trees planted in gardens from residential, unpolluted areas. However, Bousquet *et al.* [4] consider that the increase of these allergy cases must be caused by the high amount of airborne pollen as a result of the Cupressaceae ornamental population increase, or because stress has changed the pollen glucoproteins, or simply, the better quality of diagnostic tests.

Moreover, cross-reactivity between different species belonging to the Cupressaceae, Taxodiaceae and Rosaceae families has been shown by a variety of different biochemical techniques [21, 22, 26, 35, 38].

Therefore, the objectives of this study are to ascertain the influence that a climatic parameter such a temperature exerts on the onset of the pollination of Cupressaceae pollen type, in order to identify the start of the dormancy period, its duration and the consequent temperature requirements. Furthermore, it is important to ascertain the base temperatures required to overcome this vegetative period in order to identify if there is a relationship between base temperature variation and the climatic area.

## MATERIALS AND METHODS

The city of Ponferrada is situated in the northwestern quarter of the Iberian Peninsula (latitude 42° 33' N, longitude 6° 35' W, altitude 541 m), biogeographically placed in

the Mediterranean region, lower supramediterranean bioclimatic stage with a lower subhumid ombroclimate [27]. It has a mean annual temperature of 13°C and 597 mm of annual total precipitation.

The meteorological data were supplied by the Ponferrada station of the Territorial Meteorological Centre of Castilla y León, situated 1 km from the sampler location.

Pollen grains were sampled by using a volumetric collector, type Hirst seven-day-recording trap (model Lanzoni-VPPS 2000), from 1 January 1996–31 December 2005 (a 10 years period). The samples were prepared and counted by the method proposed by the Spanish Aerobiology Network [7]; according to this, the pollen data is expressed as grains per cubic meter of air.

In this paper, we determined the MPP in accordance with the criteria used by Andersen [1], corresponding to 95% of total pollen caught. In order to calculate the freezing requirement, we used the thermal time model proposed by Aron [3], based on the accumulation of chilling hours between 0-7.2°C. This method considers the maximum and minimum temperatures and the length of the chilling period:

$$N = 801 + 0.253B + 7.57B^2 \times 10^{-4} - 6.51B^4 \times 10^{-10} - 11.44T_{i_{min}} - 3.32T_{i_{max}}$$

where  $N$  = number of chilling hours during the period,

$$B = 24D [(A - T_{i_{min}})/(T_{i_{max}} - T_{i_{min}})]$$

where  $T_{i_{min}}$  and  $T_{i_{max}}$  are the average minimum and maximum temperatures during the study period respectively, and  $D$  is the length of the study period in days.

In this study, the value  $A$  in the  $B$  formula has been substituted by the different base temperatures (6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4 and 7.5°C) in order to identify which is the more effective base temperature for chilling accumulation.

We began calculating the accumulation of chilling hours (CH) from the moment of the year when the mean temperature dropped below 12.5°C to the day when a change in temperature trend was observed. The value of 12.5 is the base temperature below which winter-flowering woody plants are considered to begin to satisfy their chilling requirement [31]. In the city of Ponferrada this base temperature is registered during October or November. This is the reason why, to calculate the chilling and heat requirements, we have used the period from 1 October–31 October in the following year.

The heat accumulation required was calculated as a function of the sum of the difference between the daily maximum temperature and the threshold temperatures (0, 1, 2, 3, 4, 5 and 6°C) from the end of the chilling period to the beginning of the pollen season. The heat requirement is expressed in growth degree days (GDD) [9].

The lowest coefficient of standard deviation was used to identify the most adequate threshold temperature in both cases.

For validation of the date obtained by these forecasting methods for the beginning of the pollen season we have used 3 periods: 2002-2003, 2003-2004 and 2004-2005.

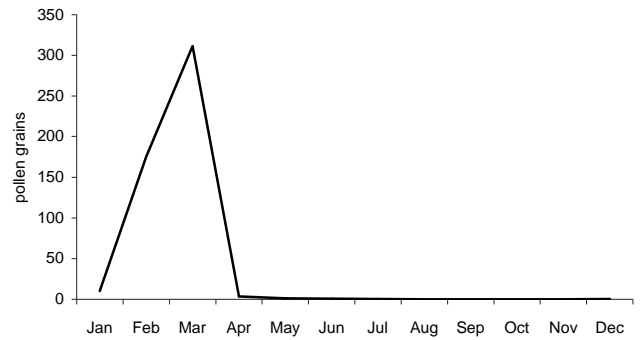
## RESULTS

Cupressaceae pollen type is detected in the atmosphere of Ponferrada mainly during the winter-spring, particularly in February and March. On average, the main pollen season starts on 11 February and ends on 20 March, with an average duration of 33 days (Tab. 1).

The total pollen index displayed a significant year-on-year variation, ranged between 35,709 pollen grains in 1999 and 2,304 in 1998. Cupressaceae pollen type accounted for between 22-65% of the total annual pollen recorded. The highest single value recorded was 6,634 pollen/m<sup>3</sup> on 11 March 1999 (Tab. 1).

For the main pollen season we have obtained an average of 44% of total annual Cupressaceae pollen type in the whole period under study.

In Figure 1 we have represented the variation of Cupressaceae pollen type concentration between January–



**Figure 1.** Cupressaceae pollen type average monthly concentration during the period under study.

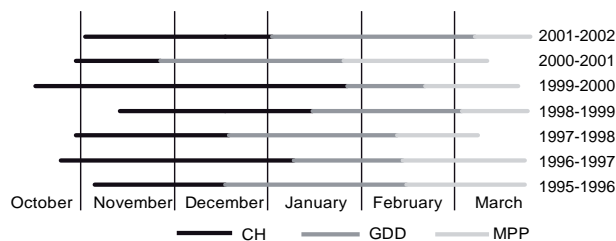
December, considering for each day the average value of the concentrations registered during the MPP. Peak concentrations were generally attained during the months of February and March.

**Table 1.** Cupressaceae pollen season characteristics, calculated according to Andersen [1] method: starting and ending date, length, average concentration during pollen season, values of peak day in pollen/m<sup>3</sup> and its date, total annual pollen, percentage of total annual Cupressaceae pollen type over annual total pollen of Ponferrada.

Pollen season	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Average
Start date	12-Feb	11-Feb	9-Feb	2-Mar	18-Feb	23-Jan	6-Mar	8-Feb	3-Feb	15-Mar	11-Feb
Final date	22-Mar	22-Mar	7-Mar	23-Mar	20-Mar	10-Mar	24-Mar	23-Mar	16-Mar	29-Mar	20-Mar
Length (days)	39	40	27	22	32	47	19	44	43	15	33
Average	85	100	85	1623	536	441	1152	383	447	420	586
Maximum (pollen/m <sup>3</sup> )	581	592	354	6634	2282	2573	4793	2735	5668	1081	2729
Maximum date	18-Feb	23-Feb	20-Feb	11-Mar	26-Feb	14-Feb	19-Mar	2-Mar	4-Mar	19-Mar	16-Mar
Total pollen	3386	4003	2304	35709	17147	20718	21894	16838	19208	6300	14751
% over the total pollen	22	38	44	65	43	46	49	43	46	43	44

**Table 2.** Chilling hours (CH) of Cupressaceae pollen type in Ponferrada during the period under study using different threshold temperatures.

Periods	Threshold temperatures (°C)											Beginning CH	Final dates	Days
	6.5	6.6	6.7	6.8	6.9	7	7.1	7.2	7.3	7.4	7.5			
1995-96	848	857	867	876	886	896	906	917	927	937	947	04-11-95	16-12-96	43
1996-97	1,151	1,149	1,144	1,135	1,123	1,108	1,087	1,063	1,033	999	959	24-10-96	07-01-97	76
1997-98	860	871	883	896	908	920	933	945	958	971	983	29-10-97	17-12-97	50
1998-99	1,042	1,017	988	957	924	887	847	805	759	709	657	12-11-98	13-01-99	63
1999-00	1,107	1,086	1,060	1,030	996	956	911	860	803	740	671	16-10-99	24-01-00	91
2000-01	832	840	847	855	862	870	878	886	894	902	911	29-10-00	25-11-00	28
2001-02	1,113	1,099	1,084	1,067	1,049	1,030	1,010	988	965	941	915	01-11-01	30-12-01	60
Mean	993	970	965	958	950	940	927	913	896	876	855			
Maximum	1,151	1,149	1,144	1,135	1,123	1,108	1,087	1,063	1,033	999	983			
Minimum	832	840	847	855	862	870	847	805	759	709	657			
Standard Deviation	143	132	120	108	96	88	84	88	101	122	150			
Coef. Std. Var. %	14.4	13.6	12.4	11.2	10.1	9.3	9.0	9.7	11.3	13.9	17.5			



**Figure 2.** Chilling hours (CH), growth degree days (GDD) and mean pollination period (MPP) for Cupressaceae pollen type in Ponferrada during 1996-2002.

The chilling accumulation period, in general, started in the second fortnight of October and continued until the end of January (Fig. 2). The best result was obtained with a threshold temperature of 7.1°C, which showed the smallest coefficient of standard deviation (9%) and an average of 927 CH (Tab. 2). The duration of chilling accumulation oscillated between 28 days in the period 2000-2001 and 91 days in 1999-2000 (Fig. 2 and Tab. 2), with an average of 59 days for the study period (Fig. 2).

Once chilling requirements were attained, heat accumulation was calculated. The smallest coefficient of standard deviation (39.1%) was obtained with a threshold temperature of 0°C, with an average of 490 GDD (Tab. 3). The duration of the heat requirements fluctuated between 24 days in the period 1997-1998 and 66 days in 2001-2002. Thus, heat requirements were achieved with an average of 45 days (Fig. 2).

Finally, we predicted the date of the beginning of the Cupressaceae pollen type season in Ponferrada for 2002-2003, 2003-2004 and 2004-2005. Results obtained for chilling (927 CH) and heat (490 GDD) requirements were used to validate models. Dates predicted were 28 January for 2002-2003, 3 February for 2003-2004 and 1 February

for 2004-2005. Afterwards, we verified the start of MPPs with the real dates obtained: 8 February in 2002-2003 (11 days later than predicted), 3 February (as predicted) and 15 March (42 days later than predicted) (Tab. 1).

## DISCUSSION

Cupressaceae pollen type is present in the city of Ponferrada (León) during the winter and early spring, and accounts for between 22-65% of the total annual pollen, depending on the study year. Thus, this pollen type is the most important in this town (see bulletins of the REA: Spanish Aerobiology Network). Most of the pollen collected in the aerobiological trap corresponds to *Cupressus* genus, abundantly represented in the study area by the species *C. arizonica* and *C. sempervirens*.

The main pollen season usually starts at the beginning of the year, depending on the temperatures in the previous period. It takes place with a slight delay with regard to other Spanish localities, such as Málaga and Vigo [29, 34].

Although in Ponferrada the MPP is not longer than 50 days, the amount of pollen is nearly 36,000 pollen grains. Throughout Spain, the length of the pollen season varies between 135-300 days, with lower Cupressaceae pollen type concentrations recorded [24, 34]. These differences may be because *Cupressus sempervirens* is the most abundant taxa and it is situated relatively close to the pollen-trap in Ponferrada. However, it is known that in spite of the proximity of these trees to the trap, a huge amount of pollen grains is lost [17]. The low concentration of pollen registered in the early years of this study could be due to the short vegetative development of the trees, as has been suggested by Pichot [28] and Andreoli & Raddi [2].

The variations of maximum temperature and relative humidity during these 9 years of study can help us to

**Table 3.** Heat requirements (HR) of Cupressaceae pollen type in Ponferrada during the period under study using different threshold temperatures.

Periods	Threshold temperatures (°C)							Beginning HR	Final dates	Days
	0	1	2	3	4	5	6			
1995-96	601	543	485	427	369	311	253	17-12-96	12-02-96	58
1996-97	371	335	299	263	227	191	155	08-01-97	11-02-97	36
1997-98	253	229	205	181	157	133	109	18-12-97	09-02-98	24
1998-99	503	457	411	365	319	273	227	14-01-99	02-03-99	46
1999-00	293	267	241	215	189	163	137	25-01-00	18-02-00	26
2000-01	655	596	537	478	419	360	301	26-11-00	23-01-01	59
2001-02	757	691	625	559	493	427	361	31-12-01	06-03-02	66
Mean	490	445	400	355	310	265	220			
Maximum	757	691	625	559	493	427	361			
Minimum	253	229	205	181	157	133	109			
Standard Deviation	192	175	158	142	125	109	92			
Coef. Std. Var. %	39.1	39.3	39.6	37.9	40.3	41.0	41.9			

understand the differences obtained with regard to the lengths and dates of the beginning and ending of the MPP [8, 23, 30].

To date, we have not found any research about predictive models with Cupressaceae pollen type, therefore, for the purpose of discussion we have used the results obtained with other winter flowering arboreal taxa.

Cupressaceae pollen type in general, especially *Cupressus sempervirens*, needs to accumulate an average of 927 chilling units. Very similar values have been obtained by *Alnus* in Perugia (Italy) (952 CH) [18], Ponferrada (León, Spain) (848 CH) [15], Ourense and Santiago de Compostela (Galicia, Spain) (816 CH and 798 CH respectively) [32]. But in Copenhagen (Denmark), this *Alnus* accumulates a greater number of chilling units (1,550 CH) to pass the dormant period [1]. Different mean temperatures may be caused of these differences. In the north of Europe this climatic factor is lower, therefore it needs to accumulate more chilling hours.

We obtained the best results with a threshold temperature of 7.1°C. However, González Parrado *et al.* [15], obtained the best result for *Alnus* with 6.5°C and Rodríguez Rajo *et al.* [32] with 5.5°C. These differences may be due to the earlier flowering of *Alnus* that occurs in December–January, in addition to the theories of another author in which the species are able to adapt to the climatic conditions of their geographic areas [13].

Once chilling requirements have been attained the heat accumulation phase starts. We obtained the best threshold temperature with 0°C, the same threshold used for *Alnus* by González Parrado *et al.* [15]. But for Cupressaceae pollen type in Ponferrada, the mean heat requirements (490 GDD) have been higher than that obtained for other taxa: *Alnus* in Ponferrada (143 GDD) [15], in Vigo (52 GDD) [34], in Perugia (42 GDD) and in Santiago de Compostela (50 GDD) [18]; *Quercus* in León needs 352 GDD, in Santiago de Compostela 420 GDD, in Barcelona 417 GDD, in Madrid 205 GDD and in Córdoba 153 GDD [13]; and *Corylus* only 55 GDD in Vigo [34]. Such differences among all these taxa may be due to the fact that these requirements are conditional on genetics, or to localities with climatic differences. García Mozo *et al.* [13] and Jato *et al.* [18], have pointed out this fact in previous studies.

In similar studies carried out previously, it was observed that the longer the chilling period the less days of heat accumulation are needed to start the flowering [3, 31]. This fact has also been observed in our work, therefore this could be the reason for the high coefficients of standard deviation obtained for the heat.

As for the validation of the predictive model used to determine the start of the pollen season, we have used 2002–2003, 2003–2004 and 2004–2005. To help us to understand the results, we have to take into account that warm temperatures during the chilling period not only signify the absence of chilling, but will reverse some of the effects of previous exposure to chilling temperatures; the negative

effect of high temperatures increases steeply between 19–21°C as was pointed out by Aron [3].

In 2004–2005, about a fortnight of high temperatures in November (nearly 19°C) were registered that prompted a suspension of chilling accumulation, which started anew in December. This fact, together with the lack of precipitation, gave us the great difference between the date predicted and that observed for the start of the Cupressaceae pollen season (43 days). In previous studies carried out with *Alnus* pollen type, a difference of 3 and 1 day was obtained, respectively, between the predicted and the flowering observed date [15, 32]. Moreover, other year-on-year differences in the pollen season start date may be due to high temperatures during the freezing period and low temperatures during heat accumulation [18].

## CONCLUSIONS

We have observed that the longer the chilling period the less days of heat accumulation are needed to start the flowering, which could explain the high coefficients of standard deviation obtained for heat.

Chilling and heat accumulation requirements of the different species of trees in the temperate area, differ as a function of local climate conditions, areas with similar mean annual temperatures displaying similar requirements. Further research is required to determine more precisely the influence of temperature on the length of the chilling and heat accumulation periods, and thus on the onset of flowering.

## Acknowledgements

The authors are grateful to the Environment Office of the Ponferrada Municipal Government. This study was partially supported by Junta de Castilla y León, project code LE044A07.

## REFERENCES

1. Andersen TB: A model to predict the beginning of the pollen season. *Grana* 1991, **30**, 269–275.
2. Andreoli C, Raddi P: Les cupressacées: genres et espèces. *Allergy Immunol* 2000, **32**, 129–131.
3. Aron R: A availability of chilling temperatures in California. *Agric Meteorol* 1983, **2**, 351–363.
4. Bousquet J, Knani J: Heterogeneity of atopy. I. Clinical and immunologic characteristics of patients allergic to cypress pollen. *Allergy* 1993, **48**, 183–188.
5. Cannell MGR, Smith RI: Thermal time, chill days and prediction of budburst in *Picea sitchensis*. *J Appl Ecol* 1983, **20**, 951–963.
6. Charpin D, Burnbaum J, Haddie E, Genard G, Toumim M, Vervollet D: Altitude and allergy to house dust mites: An epidemiologic study in primary school children. *J Allergy Immunol* 1990, **85**, 185.
7. Domínguez E, Galán Soldevilla C, Villamandos de la Torre F, Infante E, García-Pantaleón F: Manejo y evaluación de los datos obtenidos en los muestreos aerobiológicos. *Monogr REA/EAN* 1991, **1**, 1–13.
8. Emberlin J, Jones S, Bailey J, Caulton E, Corden J, Dubbels S, Evans J, McDonagh N, Millington W, Mullins J, Russell R, Spencer T: Variation in the start of the grass pollen season at selected sites in the United Kingdom 1987–1992. *Grana* 1994, **33**, 94–99.
9. Faust M: *Physiology of Temperate Zone Fruit Trees*. John Wiley & Sons, New York 1989.



10. Fornaciari M, Orlandi F, Romano B: Phenological and aeropalynological survey in an olive orchard in Umbría (Central Italy). *Grana* 2000, **39**, 246-251.
11. Frenguelli G, Bricchi E: The use of the pheno-climatic model for forecasting the pollination of some arboreal taxa. *Aerobiologia* 1998, **14**, 39-44.
12. Galán C, García-Mozo H, Cariñanos P, Alcázar P, Domínguez-Vilches E: The role of temperature the onset of the *Olea europaea* L. pollen season in Southwestern Spain. *Int J Biometeorol* 2001, **45**, 8-12.
13. García-Mozo H, Galán C, Aira MJ, Belmonte J, Díaz de la Guardia C, Fernández D, Gutiérrez AM, Rodríguez FJ, Trigo MM, Domínguez-Vilches E: Modelling start of oak pollen season in different climatic zones in Spain. *Agric For Meteorol* 2002, **110**, 247-257.
14. García-Mozo H, Galán C, Gómez-Casero MT, Domínguez-Vilches E: A comparative study of different temperature accumulation methods for predicting the start of the *Quercus* pollen season in Córdoba (South West Spain). *Grana* 2000, **39**, 194-199.
15. González-Parrado Z, Fuertes-Rodríguez CR, Vega-Maray AM, Valencia-Barrera RM, Rodríguez-Rajo FJ, Fernández-González D: Chilling and heat requirements for the prediction of the beginning of the pollen season of *Alnus glutinosa* (L.) Gaertner in Ponferrada (León, Spain). *Aerobiologia* 2006, **22**, 47-53.
16. Hänninen H: Modelling bud dormancy release in trees from cool and temperature regions. *Acta For Fenn* 1990, **213**, 1-47.
17. Hidalgo PJ, Galán C, Domínguez E: Pollen production of the genus *Cupressus*. *Grana* 1999, **38**, 296-300.
18. Jato MV, Frenguelli G, Rodríguez FJ, Aira MJ: Temperature requirements of *Alnus* pollen in Spain and Italy (1994-1998). *Grana* 2000, **39**, 240-245.
19. Kramer K: Selecting a model to predict the onset of growth of *Fagus sylvatica*. *J Appl Ecol* 1994, **31**, 172-181.
20. Laaidi M: Forecasting the start of the pollen season of Poaceae: evaluation of some methods based on meteorological factors. *Int J Biometeorol* 2001, **45**, 1-7.
21. Landsberg JJ: Apple fruit bud development and growth; analysis and an empirical model. *Ann Bot* 1974, **38**, 1013-1023.
22. Luengo O, Cárdbaba B, Lahoz C: Biología molecular y polinosis: antígenos recombinantes de pólenes. In: Valero Santiago AL, Cadahía García A (Eds): *Polinosis II. Polen y Alergia*, 51-82. Laboratorios Menarini S.A., Barcelona, 2005.
23. Mandrioli P: Biometeorology and its relation to pollen count. In: Bohem & Leushner (Ed.): *Advances in Aerobiology*, 37-41. 3rd. International Conference on Aerobiology. Basel, Switzerland 1987.
24. Méndez J: *Modelos de comportamiento estacional e intradiurno de pólenes y esporas de la ciudad de Orense y su relación con los parámetros meteorológicos*. Tesis Doctoral, Universidad de Vigo 2000.
25. Muranaka M, Suzuki S, Koizumi K, Takafuji S, Miyamoto T, Ike-mori R: Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. *J Allergy Clin Immunol* 1986, **77**, 616-623.
26. Panzani R, Yasueda H, Shimizu T: Cross-reactivity between the pollens of *Cupressus sempervirens* and of *Cryptomeria japonica*. *Ann Allergy* 1986, **57**, 26-30.
27. Penas Merino A, García González M, Herrero Cembranos L, Puente García E: Pisos bioclimáticos. Ombrotipos. In: Gallego Valcarce E, Alonso Herrero E, Penas Merino A (Eds): *Atlas del Medio Natural de la provincia de León*, 23-24. Instituto Tecnológico Geominero de España, Diputación de León 1995.
28. Pichot C: Variabilité de la pollinisation et du pollen chez les ciprés. *Allerg Immunol* 2000, **32**, 132-133.
29. Recio M: *Análisis polínico de la atmósfera de Málaga (1991-1994). Relación con los parámetros meteorológicos*. Tesis Doctoral, Universidad de Málaga 1995.
30. Richard P: Contribution aéropalynologique a l'étude de l'action des facteurs climatiques sur la floraison de l'orme (*Ulmus campestris*) et de l'if (*Taxus baccata*). *Pollen et Spores* 1985, **27**, 53-94.
31. Richardson EA, Seeley SD, Walker DR: A model for estimating the completion of rest for "Redhaven" and "Elberta" peach trees. *Hortscience* 1974, **9**(4), 331-332.
32. Rodríguez-Rajo FJ, Dacosta N, Jato V: Environmental factors affecting the start of pollen season and concentrations of airborne *Alnus* pollen in two localities of Galicia (NW Spain). *Ann Agric Environ Med* 2004, **11**, 35-44.
33. Rodríguez-Rajo FJ, Méndez J, Jato V: Influencia de la temperatura en la floración de *Quercus* en el sur de Galicia (Ourense y Vigo, 1994-98). *Acta Bot Malacitana* 2000, **25**, 153-163.
34. Rodríguez-Rajo FJ: *El polen como fuente de contaminación ambiental en la ciudad de Vigo*. Tesis Doctoral, Universidad de Vigo 2000.
35. Schwietz LA, Goetz DW, Wisham BA: Cross reactivity among conifer pollens. *Ann Allergy Asthma Immunol* 2000, **84**, 87-93.
36. Snyder RL, Spano D, Cesaraccio C, Duce P: Determining degree-days thresholds from field observations. *Int J Biometeorol* 1999, **42**, 177-182.
37. Spano D, Cesaraccio C, Duce P, Snyder R: Phenological stages of natural species and their use as climate indicators. *Int J Biometeorol* 1999, **42**, 124-133.
38. Suárez-Cervera M, Takahasi Y, Vega-Maray A, Seoane-Camba JA: Immunocitochemical location of Cry j 1, the major allergen of *Cryptomeria japonica* (Taxodiaceae) in *Cupressus arizonica* and *Cupressus sempervirens* (Cupressaceae) pollen grains. *Sex Plant Reprod* 2003, **16**, 9-15.
39. Suárez-Cervera M, Castells T, Vega-Maray A, Fernández-González D, Moreno-Grau S, La Hoz C, Seoane-Camba JA: *The role of Cup a 3, a pollen thaumatin PR5-like protein, in Cupressus arizonica pollen grains*. Autumn Meeting, Progress in Palynology, Linnean Society. London, October 2006, oral communication.
40. Wielgolaski FE: Starting dates and basic temperatures in phenological observations of plants. *Int J Biometeorol* 1999, **42**, 158-168.