

Differences in atmospheric trees pollen seasons in winter, spring and summer in two European geographic areas, Spain and Italy

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Received: 26 June 2012 / Accepted: 26 September 2012 / Published online: 17 October 2012
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Abstract The global climate change reported over recent years may prompt changes in the atmospheric pollen season (APS). The aim of this study is to evaluate the possible impact provoked by meteorological conditions variations at different seasons of the year or different geographical areas on APS. *Alnus*, *Betula* and *Castanea* atmospheric pollen seasons and trends during the last 17 years at Ourense and Vigo (Galicia—NW Spain) and Perugia (Italy) were analysed. Possible incidence of the meteorological trends observed in the different cities on the atmospheric pollen seasons and the chill and heat requirements

were evaluated. Pollen data from Ourense, Vigo and Perugia (1995–2011) were used. Pollen sampling was performed using LANZONI VPPS 2000 volumetric traps (Hirst in Ann Appl Biol 36:257–265, 1952), placed on top of different buildings at a similar height from the ground. Several methods, dates and threshold temperatures for determining the chill and heat requirements needed to trigger flowering were tested. Different temporary order in the pollination sequence was observed between the three pollen types studied in the three sites. *Alnus* flowers few days in advance in Ourense respecting to Vigo and 1 month earlier than Perugia. The *Betula* flowering start date in Ourense and Vigo is almost simultaneous, taking place only 5 days in advance with respect to Perugia. Finally, scarce differences in the APS onset of *Castanea* were detected between the three cities. The variations observed among the two areas (Umbria, Italy and Galicia, Spain) in the onset of pollen season in the winter or spring flowering trees could be explained by differences in the thermal requirements needed for flowering as consequence of the climatic conditions recorded during the previous period to flowering. The length of the chilling and heat period as well as the thermal requirements obtained showed differences between geographical areas. The chill requirements accumulated were higher in Perugia than Ourense and Vigo. By contrary, the lowest heat accumulation was achieved in Perugia. The observed trends in the APS characteristics and the weather-related parameters were not homogeneous both in the pollen types and

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sites. The pollen index of *Betula* and *Castanea* pollen in Ourense shows a significant trend to increase.

Keywords *Alnus* · *Betula* · *Castanea* · Climate change · Thermal requirements · Atmospheric pollen season

1 Introduction

According to the Intergovernmental Panel on Climate Change (IPCC), during the last hundred of years (1906–2005), a global surface air temperature average increase higher than 0.7 °C was observed, which is likely to continue throughout the 21st century (Meehl et al. 2007). The rate of the increase projected varies between 1.8 and 4 °C (2090–2099) depending on the geographical area or the scenario considered into the models.

The response of the phenological events to warming in Europe is well documented (Menzel and Fabian 1999; Chmielewski and Rötzer 2001; Fitter and Fitter 2002; Nordli et al. 2008; Ahas et al. 2002; Menzel et al. 2006a, b). Even though temperature trends display regional differences, with a not uniform influence on ecological processes, an earlier spring and latter autumn start date of the phenological events were observed. The most important factor influencing phenological events is considered to be the temperature, mainly when water availability is not a limiting factor (Sparks et al. 2000). Phenological response to temperature is a complex process as consequence to its interaction with other local diverse factors such as precipitation, photoperiod, water availability, diseases, soil factors, genetic, wind, age of the plants, etc. (Wielgolaski 2001, 2003; Ibañez et al. 2010). Thus the species response to temperature changes could significantly vary among sites (Menzel et al. 2006a; Gordo and Sanz 2009; Primack et al. 2009). Different effects of temperature and weather-related parameters trends through the year could provoke different responses on each phenological event (Gordo and Sanz 2009). Menzel et al. (2006a) noted that earlier species and phases had the strongest reactions and they better indicated changes in temperature. In general, a strong trend along the yearly calendar progression from the advancement to the delay of the start date of the phenological events was detected (Menzel et al. 2006b).

One of the major effects of shifts in plant phenology is going to be their impact on the length of the growing season; phenological changes were recently studied for different tree species in France predicting a marked gradual increase of the growing season from northeast to southwest areas (Lebourgeois et al. 2010).

In temperate areas, the trees phenological behaviour is regulated by the chill and heat thermal requirements completion, which could be also affected by the climate change. This regulation is such that a period with chilling temperatures followed by a period with forcing temperature induces budburst. The latency breaks when buds are exposed to cool temperatures over a certain period of time, during which plants complete their chilling requirements (Kramer 1994). Thereafter, buds remain inactive as a result of unfavourable environmental conditions (quiescence), but their exposure to increasing temperatures allows them to overcome the heat requirements (“forcing units”) that force buds to open and therefore flower (Jato et al. 2004; Rodríguez-Rajo et al. 2006). Chilling and heat requirements, as well as a predetermined threshold temperature, are specific to each species, bioclimatical area and population (Chuine and Cour 1999; Wielgolasky 1999). Furthermore, the climate change could not be uniform through the year, and therefore, the phenological responses to changes in the periods in which chilling and heat accumulated are unlikely to be uniform.

Climate change also affects human health through diverse pathways, including allergies to pollen grains, by inducing changes in the atmospheric pollen season (earlier onset, length and pollen index) or in the allergenic plants distribution areas (Cecchi et al. 2010). Changes in the APS in Europe are likely to be more evident in early spring flowering tree species. A number of European areas have reported an earlier onset in tree species such as alder, hazel, birch, olive or oak (García-Mozo et al. 2006; Frei and Gassner 2008; Emberlin et al. 2002; Galán et al. 2005). However, evidence is less clear for late-spring species (Emberlin et al. 2002). Different behaviour was noted in different geographical areas signalling a delay in the onset of the pollen season in different species (Recio et al. 2009; Jato et al. 2009).

In temperate climates, it is generally agreed that temperature is the most important variable influencing the production and release of pollen. Moreover, there are evidences that phenological shifts have already modified the annual carbon cycle of terrestrial

ecosystems provoking an increase in net ecosystem productivity associated with a longer growing season (Richardson et al. 2009; Linderholm 2006). Therefore, changes in the pollen production could be induced, such as the trend towards a higher daily mean pollen concentrations recently signalled for birch (Frei and Gassner 2008; Spieksma et al. 2003) or the tendency towards a more severe grass pollen seasons noted in North London (Emberlin and Smith 2006).

This paper sought to evaluate the impact produced by variations of weather conditions at different seasons of the year or different geographical areas in the phenological event of flowering. The analysis of recent trends in airborne pollen counts for three plants was conducted: *Alnus* which flowers in early winter; *Betula* in the late winter or early spring; and finally *Castanea* which flowers in early summer.

2 Materials and methods

2.1 Study area

The survey was conducted in three areas with different geographic and climatic conditions (Fig. 1; Table 1). Perugia (Umbria—Central Italy) is located approximately 90 km inland on the west-facing slopes of the Apennines, 493 m above sea level,

overlooking the valley of the Tiber River to the southwest and Lake Trasimeno to the northwest. The climate is submediterranean. The two Spanish cities are located in Galicia (NW Iberian Peninsula). Despite the general Galician climate is influenced by the Atlantic Ocean, there are important differences between both cities. Ourense is located at 120 m above sea level 100 km inland Atlantic Ocean. The climate is considered as Oceanic with stronger Mediterranean influence, showing higher temperature oscillations (summer–winter and day–night) and lower annual rainfall than Vigo. Vigo has a temperate maritime climate, and it is situated at sea level on the shore of the Vigo estuary.

2.2 Aerobiological monitoring

Volumetric Hirst design spore traps (Hirst 1952) were used in all cities (model LANZONI VPPS 2000). They were located at similar high from the ground (15–20 m) in the Agricultural Faculty in Perugia, in the Sciences Faculty in Ourense and in the City Hall building in Vigo.

Melinex tape coated with a 2 % silicone solution was used as pollen-trapping surface. The exposed tape was cut into seven pieces, which were mounted on separate glass slides. Pollen grains were counted following the model proposed by the Spanish Aerobiological Network (R.E.A.), based on four longitudinal transects along the slides (Galán et al. 2007). The *Alnus*, *Betula* and *Castanea* pollen content in the air was expressed as daily mean pollen concentrations per cubic metre of air (pollen grains/m³).

The period corresponding to the APS (Jato et al. 2006) in every year and sampling station was determined by means of the criteria proposed by Andersen (1991) and Torben (1991). According to them, the start day is considered when the accumulated sum of pollen reached 2.5 % of the total annual (APS onset) and the end date (APS end) the day when the 97.5 % is reached. The APS is therefore considered as the period during which 95 % of the total annual is recorded. The date in which the highest annual daily mean pollen concentration was recorded was considered as the peak date. The running 3 days average of the daily mean pollen concentration for each year was used to obtain the all years daily mean pollen concentration average. The Pollen Index as total annual pollen registered for each pollen type was considered.

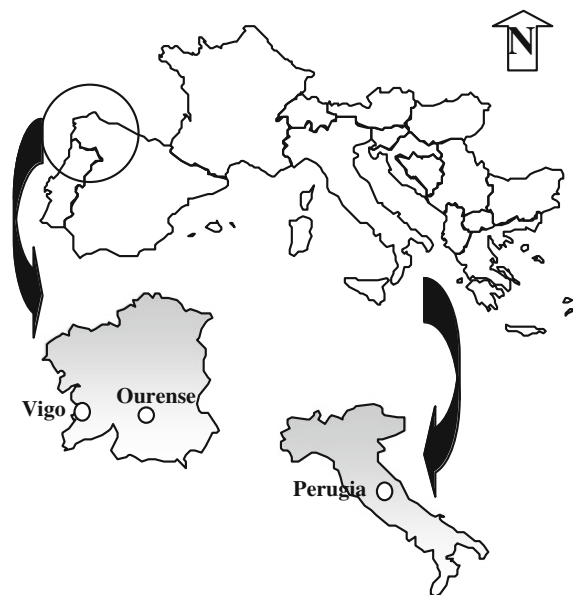


Fig. 1 Geographical location of study sites

Table 1 Biogeographical and climatic characteristics of study sites (meteorological data referred to the last 30 years)

Biogeographic characterisation					
Perugia	Mediterranean reg. Apennino-Balkan Prov. Apennine Sector				
Ourense	Mediterranean reg. Carpetano-Iberico-Leonesa Prov. Orensano-Sanabriense Sector. Orensano Subsector				
Vigo	Eurosiberian reg. Cantabro-Atlántica Prov. Galaico-Portugués Sector. Miñense Subsector				
Climatic characterisation					
	Average annual temperature	Annual maximum temperature average	Annual minimum temperature average	Annual rainfall	Geographic location
Perugia	14.2	17.1	9.2	877	43°06'N–12° 23'E
Ourense	14.8	20.9	8.6	873	42°20'N–7°52'W
Vigo	14.1	18	10.2	1,683	42°14'N–8°44'W

2.3 Pollen types selected

Daily mean pollen concentrations recorded from 1995 to 2011 (17 years) corresponding to three arboreal species flowering at different seasons of the year were utilised in this survey: in *Alnus*, which flowering occurs at the end of winter; *Betula*, that flowers at the beginning of spring; *Castanea*, whose flowering takes place during summer.

Alnus pollen sources in both Galician cities are mainly *Alnus glutinosa* (L.). Gaertner trees, making up the dominant riverside forests, accompanied by *Betula pubescens* Ehrh. (= *Betula alba* L.) and *Salix atrocinerea* Brot. Other alder species are present as ornamental trees in the gardens and streets in both cities. In Perugia (Italy) the sources of *Alnus* pollen are mainly trees of *A. glutinosa* (L.), *A. incana* (L.) Moench and *A. cordata* (Loisel.) Desf., mainly utilised as ornamental trees. *Alnus* in addition with *Corylus* (in Galicia and Perugia) and *Fraxinus angustifolia* (in Galicia) are the first species to flower every year.

Birch is represented in Galicia by *B. alba* L. (Moreno 1990). As dominant tree species, it forms altimontane oro-Cantabrian acidophilic forests, with a clearly Euro-Siberian distribution. It is found at altitudes of over 1,150 m being the last tree formation of the altitudinal sequence, with montane thermo-climates and hyper-humid ombro-climates (Izco 1994). In Galicia's mountains and foothills, non-climatic birches are found in place of montane oak groves, growing on acidic soils at heights of between 600 and 1,100 m. Birch may also form part of riparian forests along with *A. glutinosa*, *S. atrocinerea*,

Frangula alnus and *F. angustifolia*. *Betula pendula* is present as an ornamental tree in the area. The most common species sources of *Betula* pollen type in the Perugia area are *B. pendula* and *B. pubescens*, growing above all in public and private gardens where are used as ornamental trees.

In Galicia, the *Castanea sativa* Miller replaces or accompanies oak in montane- and hill-belt vegetation series of the Galaico-Asturiano Sector (*Blechno-Querceto roboris sigmetum*). This specie forms part of the acid-loving hill-belt series of the Galaico-Portugues Sector (*Rusco-Querceto roboris sigmetum*), together with *Quercus robur*, *Q. pyrenaica*, *Q. suber*, *Laurus nobilis* and *Ilex aquifolium* (Rivas Martínez, 1987). Currently, chestnut woods are principally found in areas with subcontinental climate especially in the province of Ourense, where chestnut woods occupy about 15,000 ha, and corresponding with about an 11 % of the total area occupied by this species in Spain. The *Castanea* pollen captured in Perugia comes from the only one species present in the area: *C. sativa*.

3 Thermal requirements

3.1 Chilling requirements

In order to calculate the chilling requirements, the model proposed by Aron (1983) based on the accumulation of chilling hours between 0 and 7.2 °C was used. It considers the maximum and minimum temperatures and the length of the chilling period:

$$N = 801 + 0.2523 B + 7.57 B^2 \times 10^{-4} - 6.51 B^4 \times 10^{-10} - 11.44 T_{i,\min} - 3.32 T_{i,\max}$$

where N = number of chilling hours during the period,

$$B = 24D (7.2 - T_{\min}) / (T_{\max} - T_{\min})$$

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

Moreover, the 7.2 °C considered in the method, threshold temperatures from 3 to 11 °C in steps of 0.5 °C were also considered.

The period during which the chill accumulation occurs has been considered by means the two criteria: (1) Fixed start date for all years (1 November), and a range of completion dates (from 15 December to 31st January, increasing the calculations 1 in 1 day. (2) Variable dates depending on the temperatures were recorded. The start date was the day on which the daily mean temperature equal to the chill threshold was registered. The completion date was the first day on which the daily mean temperature reached the minimum value and started to follow a positive trend (usually it occurs at the end of December or beginning of January in Ourense and Vigo, and during the second fortnight of January in Perugia). This completion date coincides with the inflexion point of a polynomial second-degree trend that followed temperatures from October of the preceding year to April of the same year as pollination.

3.2 Heat requirements

Different methods have also been considered to calculate the heat accumulation:

1. Rickman et al. (1983) method, applying the following formula for the considered period

$$^{\circ}D = \sum [(T_{\max} + T_{\min}) \times 0.5] - T_h$$

T_h = threshold temperature from which is considered the plant accumulates heat.

2. Zalom et al. (1983) method, which use daily maximum and minimum temperatures. The heat ($^{\circ}D$) is calculated according to the following premises:

$$^{\circ}D = 0; \text{ if } T_h \geq T_{\max}$$

$$^{\circ}D = [(T_{\max} - T_h)/2] / ((T_{\max} - T_h)/(T_{\max} - T_{\min})); \text{ if } T_{\min} < T_h < T_{\max}$$

$$^{\circ}D = T_{\text{med}} - T_h; \text{ if } T_h \leq T_{\min}$$

3. Chuine and Cour (1999) method, in this case Forcing units:

$$R_f(x_t) = 0; \text{ if } x_t \leq 0$$

$$R_f(x_t) = 28.4 / (1 + e^{-0.185(x_t - 18.4)}); \text{ if } x_t > 0$$

R_f = accumulated forcing degree days; x_t = daily mean temperature.

4. $^{\circ}D = \sum T_{\max}$ method, that used the sum of the daily maximum temperature.
5. $^{\circ}D = \sum (T_{\max} - T_h)$ method, that used the difference between the daily maximum temperature and threshold temperature.
6. According to Cesaraccio et al. (2004), the heat accumulation was calculated in relation to the daily maximum and minimum temperatures recorded. The terms and formulas are applied as follows:

$$Ca = T_{\text{med}} - T_h; \text{ if } 0 \geq T_h \leq T_{\min} \leq T_{\max}$$

$$Ca = (T_{\max} - T_h)^2 / 2(T_{\max} - T_{\min}); \text{ if } 0 \geq T_{\min} \leq T_h \leq T_{\max}$$

$$Ca = 0; \text{ if } 0 \leq T_{\min} \leq T_{\max} \leq T_h$$

$$Ca = 0; \text{ if } T_{\min} < 0 \leq T_{\max} \leq T_h$$

$$Ca = (T_{\max} - T_h)^2 / 2(T_{\max} - T_{\min}); \text{ if } T_{\min} < 0 < T_h < T_{\max}$$

Ca = anti-chill days.

Temperature threshold is considered to be from 3 to 7.5 °C, in steps of 0.5 °C in all cases.

The following possibilities have been considered for the period of time to calculate the heat: (1) Taking into account fixed dates and without considering the accumulated chilling: (a) From the 15th of December in the case of *Alnus* and (b) From the 1st of January for *Betula* and *Castanea*. (2) From the following date when the chill requirement has been completed. The APS onset date or the day when maximum daily mean pollen concentration was reached has been considered in all cases as the last date to heat accumulation.

3.3 Weather-related parameters

The meteorological data used (daily mean, maximum and minimum temperature and rainfall) were provided by the Agricultural Faculty in Perugia and by the Spanish Meteorological Agency (AEMET) in Galicia. All weather stations are located closer than 1 km to the spore traps. With the aim to study the tendencies of the meteorological parameters, a total of 84 variables were calculated in each city:

- Annual mean, maximum and minimum temperature and rainfall.
- Monthly average daily temperatures (maximum, minimum and mean).
- Monthly sum of daily rainfall and number of rainy days from January to December.
- Seasonal averages for the above-mentioned parameters.

3.4 Statistical treatment

Linear regression models were developed in order to detect possible trends in the main pollen behaviour characteristics over recent years. The SPSS 17.0 statistical software package was used.

4 Results

4.1 Atmospheric pollen season characterisation

The onset dates, end dates, length and pollen index (considered as the annual total pollen amount recorded) were the parameters utilised to characterise the APS for each studied pollen type at the three monitoring sites (Table 2).

Alnus: Taking into account the all years' average of the daily mean pollen concentrations, the *Alnus* APS started in Ourense during the first days of January. One month of delay was detected in Perugia in relation to Ourense and few days in relation to Vigo (Fig. 2). Similar length of the APS at the three sites was observed (around 50 days). The highest pollen index was recorded in Ourense, where both the annual total *Alnus* pollen and the daily mean pollen concentrations were higher than in Perugia and Vigo. The lowest values were registered in Perugia.

Betula: The average onset of the APS takes place at the end of March in Ourense and Vigo (only 1 day of difference as average between the two cities). A delay of 6 days was observed for Perugia in relation to Vigo and five to Ourense (Fig. 2). The length of the APS was longer in Ourense and Vigo (38 and 42 days respectively) than Perugia (29 days). As well as in the case of *Alnus*, the pollen index of APS was maximum in Ourense (where daily mean pollen concentrations and annual total pollen were the highest) and minimum in Perugia.

Castanea: The start of APS was almost coincident in the three cities. It started first in Vigo showing an advance of 4 and 6 days respectively in relation to Perugia and Ourense (Fig. 2). APS length is shorter in Perugia, and the pollen index is maximum in Ourense and minima in Perugia.

5 Thermal requirement

5.1 Chilling units

Taking into account the lowest standard deviation coefficients obtained in each city, 973 chilling units were accumulated in Perugia from 4th December to 19th January by using a threshold temperature of 3.5 °C and the Aron method, 774 chilling units from 5th December to 1st January ($T_h = 4.5$ °C) in Ourense and 733 were accumulated in Vigo from 30th November to 26th December ($T_h = 7.5$ °C). The lowest standard deviation coefficient was obtained for Vigo (Table 3). When the calculation was conducted using a fixed threshold temperature (7.2 °C) and a previously established start date (1st November) or end date (between 15th December and 31st January), the lowest standard coefficient for each city was noted when ending dates were considered during the second fortnight of December in Ourense and Vigo and at the beginning of January in Perugia (Table 3). Vigo is the city that showed the lowest standard deviation coefficients. In every case, the highest chilling requirements were accumulated in Perugia and the lowest in Vigo (Table 3).

5.2 Heat requirement

The lowest standard deviation coefficients were obtained for *Castanea*. This is the taxon whose flowering period occurs latter in the three cities. In

Table 2 *Alnus*, *Betula* and *Castanea* APS characteristics for the three study sites

	<i>Alnus</i>			<i>Betula</i>			<i>Castanea</i>		
	Perugia	Ourense	Vigo	Perugia	Ourense	Vigo	Perugia	Ourense	Vigo
Onset (date)									
Average	7 Feb	8 Jan	15-Jan	2-Apr	28-Mar	27-Mar	12-Jun	14-Jun	8-Jun
Minimum	1/28	12/20	12/19	3/19	3/12	3/11	6/6	6/1	5/25
Maximum	3/10	1/20	1/2	4/15	4/12	4/10	6/22	6/22	6/24
End (date)									
Average	26-Mar	25-Feb	5-Mar	1-Apr	5-May	7-May	22-Jul	31-Jul	10-Aug
Minimum	3/9	2/12	2/19	3/19	4/16	4/21	7/2	7/18	7/12
Maximum	4/19	3/22	3/26	4/15	5/23	5/23	8/26	8/10	9/10
Peak (pollen/m ³ and date)									
Average	41	227	112	29	284	241	72	143	93
Minimum	14 (2/27/2007)	72 (1/21/1998)	22 (2/16/1995)	3 (4/15/2005)	37 (3/27/98)	27 (3/18/2000)	4 (6/6/09)	45 (7/12/98)	22 (6/30/04)
Maximum	119 (2/14/1997)	534 (1/27/1997)	245 (1/28/1997)	58 (3/31/2002)	669 (4/27/04)	814 (28/4/2004)	149 (6/28/97)	362 (06/29/2011)	263 (06/29/11)
Length (days)									
Average	48	47	50	29	38	42	40	46	63
Minimum	23 (2005)	34 (2008)	33 (2002 y 2008)	12 (2003)	19 (2011)	26 (2010)	22 (2003)	33 (1999)	24 (2003)
Maximum	78 (1996)	70 (2000)	62 (1995)	44 (2004)	71 (2000)	72 (2000)	81 (2009)	71 (2011)	96 (2007)
Severity (annual pollen and year)									
Average	260	1896	862	152	1,860	1,166	498	1,258	846
Minimum	83 (2007)	645 (2001)	158 (1995)	19 (2005)	236 (2000)	157 (1998)	39 (2009)	450 (1998)	159 (2004)
Maximum	447 (1997)	4,526 (2008)	1,799 (2011)	346 (2009)	4,185 (2008)	2,608 (2004)	1,201 (1999)	2,493 (2011)	1,655 (2008)

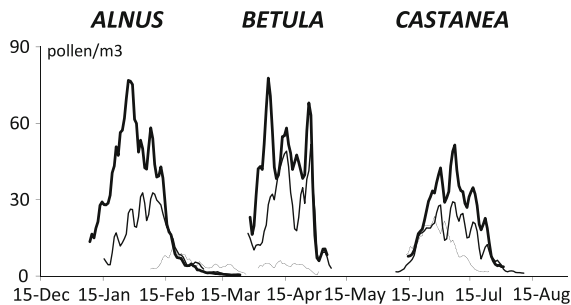


Fig. 2 *Alnus*, *Betula* and *Castanea* APS average in Perugia (grey line), Ourense (thick line) and Vigo (medium line)

Table 3 Accumulated chilling calculated with different threshold temperatures and dates in the three study sites

		Chilling units			
	Method	Date	T_h	CU	St. dv. c.
Perugia	Aron (variable T_h)	12/4–1/19	3.5	973	16.6
Ourense		12/5–1/1	4.5	774	10.7
Vigo		11/30–12/26	7.5	733	9.7
Perugia	Aron ($T_h = 7.2$ °C)	11/1–1/2	7.2	964	15.7
Ourense		11/1–12/15	7.2	756	14.4
Vigo		11/1–12/19	7.2	666	4.7
Perugia	Aron ($T_h = 7.2$ °C)	11/1–12/31	7.2	958	16.4
Ourense		11/1–12/31	7.2	834	20.2
Vigo		11/1–12/31	7.2	670	9.2

T_h threshold temperature, CU chilling units, St. dv. c. standard deviation coefficient

general, whatever the method and city considered, the lowest standard deviations coefficients were obtained when 16th December was considered as the start date for *Alnus* and *Betula* heat accumulation, while the second fortnight of January or the first fortnight of February for *Castanea*. The first day of the APS or the peak date was considered as the end date to heat accumulation.

In most cases, the lowest standard deviations coefficients were achieved considering the sum of daily maximum temperatures, the method proposed by Zalom et al. (1983) and a threshold temperature from 3 to 5.5 °C, depending of the city or pollen type considered (Table 4). Therefore, in order to compare the heat accumulated between cities and pollen types, the values obtained with the Zalom et al. (1983)

method were considered (Table 4). In this case, the best results were obtained considering the following day after the completion of chilling requirement as the starting date for heat accumulation, a threshold temperature of 5.5 °C, and both APS onset and peak date as the end of heat accumulation. Whatever the methods used the lowest heat accumulation was achieved in Perugia, with the exception of the *Alnus*' APS (Table 4). Depending on the pollen types, there were small differences between Ourense and Vigo. Higher heat accumulation for *Alnus* was observed in Vigo than Ourense, while by the contrary, it was higher in Ourense than Vigo for *Betula* and *Castanea* (Table 4).

6 APS trends

6.1 APS onset

A slight and not significant trend towards a latter start date of *Alnus* and *Betula* APS was observed at all three sites. The same trend, but significant in this case, was detected for *Alnus* peak date in Perugia, and a significant tendency to an earlier *Castanea* peak date was noted in the same city (Table 5).

6.2 Pollen index

A significant trend to an increase of the annual total pollen and the annual maximum daily mean pollen concentrations of *Betula* and *Castanea* were observed in Ourense. The same significant tendency was detected for *Castanea* annual total pollen and its peak value in Vigo. In Perugia, a significant trend to a decrease of the annual *Alnus* and *Castanea* total pollen was noted (Table 5).

6.3 Length

Only for *Castanea* pollen in Ourense was detected a trend to extend the length of the APS ($\text{adj } R^2 = 0.256$; $p < 0.05$).

7 Thermal requirements trends

7.1 Chilling

Even if a slight trend to an increase of the accumulated chilling in Vigo and a decrease in Ourense and Perugia was obtained, no significant trends was noted.

Table 4 Heat accumulated calculated by different method, threshold temperature and dates in the three study sites

Site	Method (T_h and start date)	Heat requirement											
		Alnus on set APS		Alnus peak date		Betula onset APS		Betula peak date		Castanea onset		Castana peak	
		HU	St. dv. c.	HU	St. dv. c.	HU	St. dv. c.	HU	St. dv. c.	HU	St. dv. c.	HU	St. dv. c.
Perugia		141		212		339		369		667		724	
Ourense	Zalom 3 °C (/12/16)	156		286		811		941		1,856		2,255	
Vigo		236		413		848		1,005		1,711		2,090	
Perugia		442	15.3	656	21.8	1108	12.7	1,225	12.5	2,623	7.9	2,914	7.3
Ourense	$\sum T_{\max}$ (16/12)	300	29.9	555	20.6	1589	9.4	1,838	11.1	3,415	3.9	4,043	5.5
Vigo		419	32.8	732	26.9	1,493	13.1	1,752	14.1	2,852	8.5	3,402	9.1
Perugia	Zalom 3.5 °C (variable)	110	44.6	180	27.7	302	12.9	332	15.7	630	8.3	687	8.1
Ourense	Zalom 4.5 °C (variable)	93	94	196	51.7	668	19.2	785	19.1	1,605	6.8	1971	9
Vigo	Zalom 5.5 °C (variable)	98	74.2	178	60.5	450	25.5	552	24.8	1,006	16.2	1,275	17.1
Perugia	Zalom 5.5 °C (variable date)	100	43.8	167	27.4	294	13.7	324	16.2	623	8.7	680	8.5
Ourense		87	95.9	184	51.9	630	18.8	739	18.7	1,500	6.7	1,844	9.2
Vigo		126	76.4	254	48.7	577	27	702	26.2	1,265	16.4	1,582	16.6

HU heat units, St. dv. c. standard deviation coefficient

Table 5 Observed APS trends in the three study sites

	APS trends								
	Perugia			Ourense			Vigo		
	Slope	Adj R^2	p	Slope	Adj R^2	p	Slope	Adj R^2	p
Annual <i>Alnus</i> pollen	−11.95	0.224	0.032	97	0.151	0.069	27.90	0.023	0.343
<i>Alnus</i> APS onset	0.21	–	0.665	0.45	0.019	0.270	0.640	0.060	0.175
<i>Alnus</i> peak value	−2.85	0.143	0.075	7.30	–	0.323	1.300	–	0.722
<i>Alnus</i> peak value date	0.98	0.205	0.039	−0.15	–	0.726	0.340	–	0.599
<i>Alnus</i> APS length	−0.05	–	0.937	−0.28	–	0.585	−0.402	–	0.432
Annual <i>Betula</i> pollen	1.29	–	0.758	164	0.474	0.001	35.40	–	0.345
<i>Betula</i> APS onset	0.51	0.048	0.198	0.63	0.049	0.197	0.37	–	0.477
<i>Betula</i> peak value	0.05	–	0.942	27	0.522	0.001	16.50	0.113	0.113
<i>Betula</i> peak value date	0.72	0.076	0.148	0.55	–	0.356	0.54	–	0.391
<i>Betula</i> APS length	−0.23	–	0.663	−0.29	0.066	0.164	−0.41	–	0.562
Annual <i>Castanea</i> pollen	−32.84	0.249	0.024	105	0.590	0.002	55	0.319	0.011
<i>Castanea</i> APS onset	−0.12	–	0.568	−0.20	0.028	0.323	−0.48	–	0.346
<i>Castanea</i> peak value	−4.12	0.147	0.072	10.9	0.322	0.010	7.40	0.334	0.015
<i>Castanea</i> peak value date	−0.82	0.270	0.019	0.18	0.016	0.569	0.00	–	0.992
<i>Castanea</i> APS length	0.86	0.058	0.197	1.06	0.256	0.022	1.32	0.068	0.171

Adj R^2 adjusted R^2 ; bold: significant level >95 %

7.2 Heat

A slight not significant trend to the increase of the heat accumulated amount for the *Alnus* and *Betula* APS

onset in Ourense and Vigo was noted. By the contrary, a slight significant tendency to the reduction of the *Castanea* heat accumulation in Ourense (Table 6) was observed. In Perugia a trend to the increase of the heat

accumulated amount, considering as start date the peak date, for the three pollen types (significant for *Alnus* and *Betula*) was noted.

8 Weather-related parameters trends

8.1 Temperature

A clear trend to the raise of the annual temperature was detected in Perugia. The increase is maximum for the annual average of the maximum temperature, while the mean and minimum temperatures noted the same tendency but with lower significance (Table 7). Similar trend was observed in Vigo, being only significant for the annual average of the daily minimum temperatures (Table 7). Finally in Ourense, a slight not significant trend to a decrease of the annual average of the minimum and mean temperatures and an increase of the maximum temperature were observed.

In order to check whether the detected tendencies are homogenous throughout the year, seasonal and monthly meteorological values were also analysed. A clear trend to the increase of the average maximum, mean and minimum temperatures during the spring and the summer, and the average of the autumn maximum temperatures were observed in Perugia (Table 7). The increase of the monthly temperature was more acute in the case of the April, July and September maximum temperatures, even though less significant values were also recorded during May, June and November. Similar trends were noted for the mean temperature registered in April, June and July. April and September were the only months showing a significant increase of the minimum temperature average trend (Table 7).

Albeit slighter and less significant, the tendency noted for the springtime temperatures in Vigo was the same. May and September were the months that showed the highest maximum temperature increase. May, June and September for the minimum temperature, and May in the case of the mean temperature (Table 7) showed significant monthly increase trends.

Any significant seasonal temperature trend was noted in Ourense. The only significant monthly trend was noted for the maximum and mean temperatures registered in September (Table 7).

8.2 Rainfall

Although a decreasing trend of rainfall has been observed in all cities, it was not significant.

9 Discussion

Alnus, *Betula* and *Castanea* are arboreal species whose flowering takes place at different moments of the year. The pollen from these species is recorded in both geographical areas, Galicia (Vigo and Ourense) and Umbria (Perugia), although its quantitative representation is diverse. The highest pollen concentrations for each pollen type were registered in Ourense while the lowest in Perugia.

Measurements of pollen emissions in the atmosphere are a useful tool to ascertain the timing of flowering (Frenguelli et al. 1992, Chuine et al. 1998; Jato et al. 2002a). In general, *Alnus* APS onset started first in Ourense (7 days before than Vigo and 1 month in relation to Perugia), *Betula* first in Vigo (1 day before than Ourense and 5 days with respect to Perugia) and finally *Castanea* also first in Vigo (5 days before than Perugia and 7 days before Ourense).

Table 6 Significant thermal requirement trend observed

	Heat requirement trends								
	Perugia			Ourense			Vigo		
	Slope	Adj R^2	p	Slope	Adj R^2	p	Slope	Adj R^2	p
<i>Alnus</i> peak zalom (16/12–5,5 °C)	5,070	0.360	0.006						
<i>Alnus</i> peak $\sum T_{\max}$ (16/12)	16.800	0.309	0.012						
<i>Betula</i> peak $\sum T_{\max}$ (16/12)	19.000	0.348	0.008						
<i>Castanea</i> APS onset $\sum T_{\max} - T_h$ (16/12–3 °C)				–11.540	0.189	0.046	–	–	–

Adj R^2 adjusted R^2 ; significant level >95 %

Table 7 Significant weather-related parameters trends observed in the three study sites

	Weather-related parameters trends								
	Perugia			Ourense			Vigo		
	Slope	Adj R^2	p	Slope	Adj R^2	p	Slope	Adj R^2	p
Annual maximum temperature	0.176	0.653	0.000	0.018	–	0.461	0.113	0.125	0.090
Annual minimum temperature	0.060	0.236	0.027	–0.017	–	0.551	0.133	0.183	0.049
Annual mean temperature	0.098	0.409	0.003	–0.009	–	0.657	0.074	0.030	0.24
Annual rainfall	2.840	–	0.815	–19.20	0.146	0.072	–31.77	0.06	0.164
AMJ—seasonal maximum temperature	0.245	0.549	0.000	0.024	–	0.616	0.162	0.194	0.044
AMJ—seasonal mean temperature	0.161	0.481	0.001	0.022	–	0.518	0.162	0.232	0.029
AMJ—seasonal minimum temperature	0.117	0.440	0.002	0.038	–	0.342	0.188	0.301	0.013
JAS—seasonal maximum temperature	0.295	0.523	0.001	0.093	0.115	0.099	0.114	0.099	0.117
JAS- seasonal mean temperature	0.174	0.350	0.007	0.034	–	0.389	0.038	–	0.515
JAS—seasonal minimum temperature	0.101	0.204	0.039	–0.002	–	0.962	0.148	0.143	0.075
OND—seasonal maximum temperature	0.112	0.188	0.005	0.024	–	0.724	0.127	0.050	0.202
April maximum temperature	0.331	0.510	0.001	0.024	–	0.829	0.186	0.059	0.178
April mean temperature	0.237	0.421	0.003	0.025	–	0.728	0.179	0.091	0.128
April minimum temperature	0.193	0.413	0.003	0.050	–	0.509	0.210	0.165	0.060
May maximum temperature	0.212	0.236	0.028	0.055	–	0.451	0.177	0.287	0.016
May mean temperature	0.123	0.112	0.103	0.011	–	0.822	0.166	0.294	0.014
May minimum temperature	0.066	0.188	0.271	–0.003	–	0.961	0.172	0.276	0.018
June maximum temperature	0.193	0.291	0.015	–0.009	–	0.932	0.124	0.068	0.162
June mean temperature	0.122	0.226	0.031	0.029	–	0.675	0.140	0.155	0.066
June minimum temperature	0.092	0.176	0.053	0.068	0.055	0.184	0.182	0.318	0.011
July maximum temperature	0.290	0.436	0.002	–0.033	–	0.705	0.042	–	0.633
July mean temperature	0.160	0.270	0.019	–0.040	–	0.524	–0.026	–	0.690
September maximum temperature	0.314	0.440	0.002	0.238	0.323	0.011	0.217	0.247	0.024
September mean temperature	0.204	0.254	0.023	0.129	0.308	0.015	0.137	0.127	0.088
September minimum temperature	0.158	0.184	0.049	0.053	0.007	0.308	0.199	0.257	0.022
November maximum temperature	0.141	0.294	0.014	0.024	–	0.724	0.127	0.050	0.202

Adj R^2 adjusted R^2 ; bold: significant level >95 %

Therefore, the differences in the timing of the APS onset among the early and latter flowering were reduced in the different areas as the year progresses.

The variations observed between both areas (Umbria and Galicia) in the onset of pollen season in the winter or spring flowering trees could be explained by differences in the thermal requirements needed for flowering as consequence of the climatic conditions recorded during the previous period to flower. Chilling accumulation is needed in order to break latency. Chilling period was longer in Perugia and shorter in Vigo (as average 2 months in Perugia, 39 days in Ourense and 21 days in Vigo, when 7 °C was considered as threshold temperature). An increase of

temperatures after the chilling period allows the plants to overcome the heat required blooming. The temperature registered during the 25–55 days before flowering, depending on the species and the biogeographical area, exerts great influence in order to overcome the heat required to flowering (Jato et al. 2004; Rodríguez-Rajo et al. 2006). In this survey, shorter period for heat accumulation was needed in Perugia for *Alnus* and *Betula* APS onset (22 and 75 days as average from the end of the chilling period respectively) than in Ourense (11 and 90 days) or Vigo (23 and 91 days). In the context of a possible global climatic warming, the studies focused on the quantification of the temperature requirements necessary to overcome the

dormancy period arouse particular interest. Knowledge of chilling temperatures in different areas can help us to explain the natural distribution of plants, as well as changes that could now be taking place (Rodríguez-Rajo et al. 2003). Surveys based on *Betula* and *Alnus* data have been carried out in Galicia and Perugia in order to determine the chilling and heat requirements needed to trigger bud development (Jato et al. 2000; 2002b; Rodríguez-Rajo et al. 2004). This subject is of particular important for birch, often found at the latitudinal or altitudinal tree line, where the effect of climate warming could be more severe (Myking and Heide 1995; Emberlin et al. 2002). The chilling or heat amount and the period required for both accumulations differ according to the climatic conditions of the areas. In colder areas, usually a higher chilling accumulation takes place, since trees need to protect for longer periods, and at the same time, the heat accumulation is lower as the chilling increases (Jato et al. 2000). In this survey, noticeable differences were observed between the accumulated chilling in the three sites. The highest chilling requirements were always recorded in Perugia, regardless of whatever method, the threshold temperature and dates considered. The most rigorous winter in Perugia favours a higher chilling accumulation, but delays of the accumulation of heat provokes a latter *Alnus* flowering than both Galician sites. At the same time, the higher quantity of chilling accumulated in Perugia shortened the heat accumulation period, causing a contraction of the *Betula* timing flowering differences observed between the Galician and Italian sites. Less variation between the chilling accumulated in the two Galician sites was noted.

In relation to the heat accumulation, lowest values were recorded in Perugia in relation to both Galician cities for the pollen types studied, considering as well as the onset of the APS and the peak date. In this case only for *Alnus* APS onset, a higher heat accumulation amount was noted in Vigo. In the three sites, the variability of the *Alnus* APS onset and the peak date is higher than the variation registered for *Betula* and *Castanea*. Indeed, the standard deviation coefficients observed were highest for *Alnus* whatever the method, threshold temperature and dates considered, and they could justify the differences observed for the heat accumulation. Moreover, as different species of *Alnus* are present in both geographical areas (Galicia and Umbria), the different heat requirement observed could be also genetically

determined. In both Galician sites, the same *Alnus* species are present in the surrounding area of the pollen traps. In this case, the differences observed in the heat accumulation for *Alnus* APS onset and peak date could be related to the variations of the chilling accumulated. Ourense noted higher chilling than Vigo while the heat accumulated was higher in Vigo, provoking a delay in the *Alnus* flowering in relation to Ourense. Other environmental factors could also influence the results observed; previous research conducted about *Betula* phenology in Galicia noted considerable differences in the onset of flowering populations located at different altitudes (Jato et al. 2002b).

The correlation test applied in order to detect the possible association between the chilling and heat requirements for each pollen type and site noted a negative correlation. Although the results were not always significant, these data reinforce the assertion that the places with colder temperatures present a higher chilling requirement and a lower heat accumulation. Most of the significant values were obtained for *Alnus* and *Betula* in Vigo, considering the method proposed by Zalom et al. (1983) or the sum of the daily maximum temperatures to determine the heat accumulation. Only in the case of *Alnus*, significant results were obtained in Ourense and Perugia. Nor in the sites neither whatever method used, significant trend was observed for *Castanea*.

For latter flowering trees such as *Castanea*, chilling seems to be less important and therefore an accurate heat accumulation completion seems to be the most important factor for the start date timing of its flowering. The quick increase of the temperatures during spring and summer registered in Perugia induces a faster heat accumulation. Furthermore, the higher chilling recorded could also influence the lower heat requirements observed for Perugia in relation to Ourense and Vigo. Finally, climatic differences could influence the lower requirement needed for *Castanea* at this geographical area.

When we compare the different methods and threshold temperatures used to calculate chilling and heat requirements specified in Sect. 2, the lowest coefficient of standard deviation was obtained with higher threshold temperatures in both Galician sites. Threshold temperatures of 3.5, 4.5 and 7.5 °C resulted in the lowest standard deviation coefficients for chilling calculation, meanwhile threshold temperatures between 3 and 5.5 °C revealed good results for

heat accumulation. Similar threshold temperatures were signalled in the previous studies conducted in the same areas for *Alnus* and *Betula* (Jato et al. 2000; Rodríguez-Rajo et al. 2003, 2004).

The observed trends in the start of the APS and in the weather-related parameters were not homogenous both in the pollen types and sites. During the studied years, only a slight trend to a delay of the *Alnus* peak date or to an advance of the *Castanea* peak date was noted for Perugia. Any significant trend was detected in Ourense and Vigo for any pollen type. In the last decades, numerous surveys were conducted in Europe to ascertain the influence of the climate change in phenological events. Changes in the temperature of the preceding months are responsible for the advance of 2.5 days °C⁻¹ as average for spring/summer phases, registering one of the highest marked variations in Spain in relation to others countries (Menzel et al. 2006a). Similar results were obtained in leaf unfolding and flowering from the beginning of the 1970s in Spain, showing a sharp advance, more acute in anemophilous than entomophilous species (Gordo and Sanz 2009).

In diverse European areas, a shift to an earlier APS onset of birch linked to an increase of the temperature was cited (Emberlin et al. 1997, 2002; Garcia-Mozo et al. 2006; Frei and Gassner 2008). An advance of birch and chestnut was also noted in a recent phenological study conducted in Spain (Gordo and Sanz 2009). A significant advance in the flowering start data was also signalled in Galicia for *Castanea*, while a not significant and negative trend was also pointed out for *Betula* (Perez Muñuzuri et al. 2009). The different source of the records (in both phenological and aerobiological data) and the dissimilarities of the years considered in the different surveys could explain the observed differences. However, the obtained results in this survey reinforce the previously obtained in Ourense and Vigo for *Alnus*, *Betula* and *Castanea* 1995–2009 APS trends (Rodríguez-Rajo et al. 2010). The start date of the APS itself is defined by the timing of the airborne pollen appearance. It could be influenced by factors affecting pollen dispersal (such as rainfall or lack of wind) and transport (long-range transport) processes and may not coincide exactly with the maturation of the flowers. Moreover, different weather-related parameters trends were observed in this survey that could also justify the slight effect on the APS onset variations. A

less marked tendency to a temperature increase in the Galician localities than Perugia was noted. Perugia showed a clear and significant trend to an increase of the annual mean temperature mainly due to the spring and summer temperature arises. Although less pronounced in Vigo and even in Ourense a not significantly increasing temperatures trend occurs during the same period or even at the end of summer and autumn. Therefore, the possible temperature trend influence on the *Alnus* and *Betula* APS onset is not possible since the pollen seasons have already started at that moment. However, the warmer spring and summer justify the advance detected in *Castanea* APS onset and its peak date, mainly in Perugia. The slight trend to a delay of the *Alnus* peak date in relation to the increased trend to the heat accumulation detected in Vigo may be due as consequence of the chilling decrease observed in the same city. In relation to the length of the pollen season only *Castanea* in Ourense noted a significant tendency to extend the APS.

Most noticeable changes were observed for the pollen index of APS, mainly for *Betula* and *Castanea* in Ourense which showed a clear increase of the annual total pollen quantity and the maximum daily mean pollen concentrations, and for *Castanea* in Vigo. Trends to an increase of the *Betula* pollen total amount were also signalled in different western European areas, in spite only the stations with lower pollen counts showed a significant tendency (Spieksma et al. 2003). By the contrary a clear decrease of the annual total pollen amount and the peak value concentrations was noted for *Alnus* and *Castanea* in Perugia. Allergies to pollen were increased in the last decades and climate change can also provoke changes in the APS which could worsen the patients symptomatology. The increase of the atmospheric CO₂ concentration could influence public health scenarios stimulating the pollen production. An increase of *Ambrosia artemisiifolia* pollen production was detected when plants were maintained at controlled increases of temperature and CO₂ (Ziska and Caulfield 2000; Rogers et al. 2006). Different tendencies in the annual total quantity were noted for different pollen types and sites (Jato et al. 2009; Recio et al. 2009; Emberlin et al. 1993, 2000; Frei 1998). In addition of the weather-related parameters, others factors (land use, forest fires, agricultural practices....) could be the cause for this change. *Betula* pollen has the highest allergological interest of the studied pollen types as it is considered to

be the main cause of pollinosis in North and Central Europe (Spieksma et al. 1995; D'Amato et al. 2007). The highest *Betula* pollen counts in Spain are recorded in Galicia, where daily mean pollen concentrations often exceed 100 pollen grains/m³ and pollen index is higher than 1,000 grains (Jato et al. 1999). The percentage of the positive reactions in the population sensitive to *Betula* pollen differs in different European countries, being a percentage of 13 % in the Ourense population (Arenas et al. 1996). Frei and Gassner (2008) signalled a remarkable increase in the annual airborne birch pollen content in the last 38 years. Chestnut pollen is not considered to be particularly allergenic, however studies conducted in Switzerland (Frei et al. 1995) and France (Sutra 1987) have indicated that a large proportion of the population is sensitive to this pollen. In Galicia, around an 8 % of the population of Ourense (Arenas et al. 1996) and the 2 % of the population of the city of Vigo (Belmonte et al. 1996) appear to be sensitised to this pollen type.

10 Conclusion

Small differences in the APS and the meteorological tendencies between pollen types and cities were observed.

The air temperature registered during the preceding period of the APS and the thermal requirement could explain the observed differences between the atmospheric pollen seasons in different pollen types at diverse geographical areas.

The detected trend to an increase of the average annual temperature in Perugia is mainly consequence of the warming registered in spring and summer which could justify its slight impact in the beginning of the pollen season of winter flowering trees and its higher influence in the summer flowering trees.

Acknowledgments The research conducted was supported by a grant from the Spanish Education Ministry for stays at foreign Universities (PR2009-0433).

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