

Assessment of *Quercus* flowering trends in NW Spain

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Abstract This paper sought to chart airborne *Quercus* pollen counts over the last 20 years in the region of Galicia (NW Spain) with a view to detecting the possible influence of climate change on the *Quercus* airborne pollen season (APS). Pollen data from Ourense, Santiago de Compostela, Vigo and Lugo were used. The *Quercus* airborne pollen season was characterized in terms of the following parameters: pollen season start and end dates, peak pollen count, pollen season length and pollen index. Several methods, dates and threshold temperatures for determining the chill and heat requirements needed to trigger flowering were applied. A diverse APS onset timing sequence was observed for the four cities as *Quercus* flowers few days in advance in Vigo. The variations observed could be related to differences in the meteorological conditions or the thermal requirements needed for flowering. Thermal requirements differed depending on local climate conditions in the study cities: the lowest values

for chilling accumulation were recorded in Vigo and the highest in Lugo, whereas the lowest heat accumulation was achieved in Vigo. Differences in APS trends between cities may reflect variations in weather-related trends. A significant trend towards rising *Quercus* pollen indices and higher maximum daily mean pollen counts was observed in Ourense, linked to the more marked temperature increase across southern Galicia. A non-uniform trend towards increased temperatures was noted over the study period, particularly in late summer and early autumn in all four study cities. Additionally, an increase in spring temperatures was observed in southwestern Galicia.

Keywords *Quercus* · Climate change · Thermal requirements · Atmospheric pollen season

Highlights Airborne *Quercus* pollen count study over the last 20 years in the NW Spain with a view to detecting the possible influence of climate change.

A trend to an increase of the *Quercus* pollen was observed in Ourense. A non-uniform heating through the year in the studied years was observed.

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Introduction

The *Quercus* genus is represented in Spain by 13 native oak taxa, species and subspecies (Pérez-Badia et al. 2013). These species are distributed along the Spanish territory being well represented on the Atlantic and Mediterranean regions. *Quercus* trees are anemophilous plants producing high quantities of pollen (Moore and Webb 1983; Tormo et al. 1996; Jato et al. 2007) which varies according to the species and years (ranging from 4,646 to 6,064 pollen grains/anther for *Quercus ilex* ssp. *ballota*, or from 2,817 to 6,264 for *Quercus rotundifolia*). Furthermore, *Quercus* is considered a moderate cause of pollinosis in various parts of Europe. Symptoms are detected over a prolonged period, due not only to the overlap of *Quercus* species airborne pollen seasons but also to cross-reactions with other pollens from *Alnus*, *Betula*, *Castanea*, *Olea* and *Poaceae* (Ickovic and Thibaudon 1991). The contribution of *Quercus* pollen to asthma morbidity has been confirmed in Atlanta, where very high daily mean *Quercus* pollen

counts are recorded (Darrow et al. 2012). In Galicia, skin sensitization to *Quercus* pollen ranging from 4 to 12 % has been reported among the pollen-allergy sufferers (Arenas et al. 1996; Ferreiro et al. 1998).

Pollen-curve analysis is of particular value for assessing the allergenic potential of *Quercus*. It also yields data useful for determining the ecological conservation status of forests and woodlands. Year-on-year changes in pollen-curve behaviour provide information on plant phenology, pollen production, plant distribution and the health of certain species (Hidalgo et al. 2008; García-Mozo 2011).

Due to the stenophilous character of the pollen season, the *Quercus* pollen curve usually represents the pollen released by all the species of a certain area. The contribution of each species is governed not only by its local abundance but also by its pollen production ability, the distance pollen source-pollen trap and the chance of pollen transport processes (Schueler and Schlünzen 2006, Jato et al. 2007). Airborne pollen counts are strongly influenced by weather conditions during pollen release and dispersal, which affect the persistence of pollen in the atmosphere by influencing pollen transport or prompting pollen washout during rainy days.

In temperate zones, the chill and heat required to release dormancy and trigger the onset of tree phenology has special importance in the onset of the atmospheric pollen season (APS). The chilling and heat periods as well as the threshold temperature below or above the chill or heat is accumulated are variable depending on the species and geographical area (Chuine and Cour 1999; Wielgolaski 1999). Many phenological and aerobiological studies were conducted in order to determine the chill and heat requirements of different species and geographical areas (Frenguelli and Bricchi 1998; Jato et al. 2000; Laaidi 2001; Rodríguez-Rajo et al. 2003, 2005; Jato et al. 2004).

According to the IPPC (2013), the global average mean surface temperature increased by about 0.85 °C (0.65–1.06 °C) between 1880 and 2012, whilst the total increase between the mean for the period 1850–1900 and the period 2003–2012 was 0.78 °C (0.72–0.85 °C). This rising trend is likely to continue throughout the twenty-first century. The response of plants to temperature changes is a complex process, involving interaction with a whole range of local 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. 2006; Gordo and Sanz 2009; Primack et al. 2009). The physiology of plants and their reproductive patterns could be altered by climate change. These changes may cause effects on the human health (including allergies to pollen grains) by inducing variations in the APS (onset, duration and severity) and changes in the distribution areas of allergenic plants (Shea et al. 2008; Cecchi et al. 2010;

Darrow et al. 2012). Moreover pollen allergenicity could be increased as some allergenic proteins (mainly pathogenesis-related proteins—PR—involved in the defence mechanism of plants) are widely expressed in the pollen grains under environmental stress conditions induced by pollution or climate change (Rodríguez-Rajo et al. 2011). An earlier onset in some trees such as alder, hazel, birch, olive or oak has been reported in different European areas (Frei and Gassner 2008; Emberlin et al. 2000; Emberlin et al. 2002; Galán et al. 2007). However, a delay in the onset of the pollen season was also signalled for other species as Urticaceae or Poaceae (Recio et al. 2009; Jato et al. 2009; Jato et al. 2012). There is evidence that changes in atmospheric composition have already led to an increase in net ecosystem productivity, associated with a longer growing season (Richardson et al. 2009; Linderholm 2006) and that increased pollen production is linked to higher CO₂ concentrations and/or temperatures (Ziska and Caulfield 2000; Ziska et al. 2003, Rogers Ch et al. 2006, Ziello et al. 2012). Trends towards higher daily mean pollen counts have been reported for a number of species (Spieksma et al. 2003; Emberlin and Smith 2006; Frei and Gassner 2008; Jato et al. 2012). Moreover, climate change may not take place at a uniform pace throughout the year, so phenological responses to changes in chilling and heat-accumulation periods are unlikely to be uniform (Jato et al. 2012).

Previous surveys were conducted in different Spanish localities in order to ascertain the atmospheric *Quercus* pollen season behaviour and to predict possible variations of its APS induced by climate change processes (García-Mozo et al. 2002; García-Mozo et al. 2006; Pérez-Badía et al. 2013). Models for forecasting the start of pollen season and the daily pollen concentrations were also tested (García-Mozo et al. 2008). The factors affecting *Quercus* pollination in different localities of Galicia (Rodríguez-Rajo et al. 2003, 2005), the phenological behaviour of the different *Quercus* species and their influence on *Quercus* pollen curves (Gomez-Casero et al. 2004; Jato et al. 2002, 2007) were also analyzed.

This paper sought to chart airborne *Quercus* pollen counts over the last 20 years in the region of Galicia (NW Spain) with a view to detecting the possible influence of climate change on the *Quercus* airborne pollen season (APS).

Material and methods

Study area

The analysis was conducted in four Galician cities (NW Spain; Fig. 1 and Table 1). The boundary between the Eurosiberian and Mediterranean regions divides Galicia into two territories: Lugo, Santiago and Vigo are located in the Eurosiberian region, and Ourense in the Mediterranean. Although the general Galician climate is influenced by the

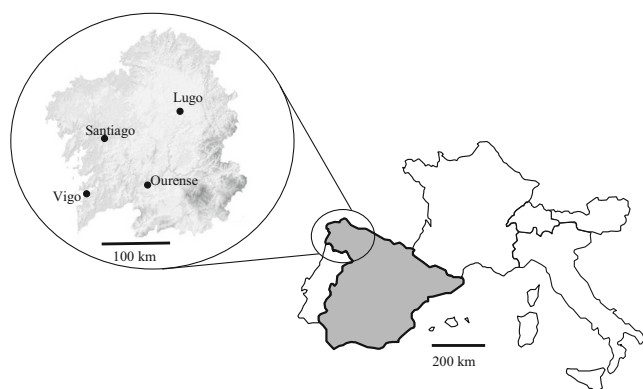


Fig. 1 Geographical location of the study sites

Atlantic Ocean, the geographic location and the topography determine the climatic and biogeographical differences between the four areas. Ourense, located at 139 m a.s.l. and 100 km inland Atlantic Ocean, shows a stronger Mediterranean influence, with higher temperature oscillations (summer-winter and day-night) and lower annual rainfall. The climate is considered as oceanic with stronger Mediterranean influence. Lugo is located at 454 m a.s.l., 100 km inland the Atlantic Ocean and the Cantabric Sea. Its climate is characterized by a cool, dry ombrothermal regime. Santiago de Compostela located at 260 m a.s.l., 60 km inland the Atlantic Ocean, presents a mild maritime climate. Finally, Vigo has a temperate maritime climate although with a certain Mediterranean aspect (Carballeira et al. 1983) and it is situated on the south bank of an estuary of the Atlantic coast.

Several types of *Quercus* forests are the predominant vegetation in Galicia. As a consequence of the special climatic and biogeographical characteristics of the four cities, there are some differences in relation to the major *Quercus* species representation in the areas surrounding the traps. In the most north-western areas, *Quercus robur* L. is the representative specie of this genus occupying the greatest geographical area. From the N-NW to the S-SW, the abundance of *Q. robur* progressively decreases and it increases the Mediterranean

influence that intensifies the presence of *Quercus pyrenaica* Willd. and *Quercus suber* L. However, the Atlantic influence makes possible the presence of *Q. robur* in all Galician territory. *Q. ilex* subsp. *ballota* (Desf.) has a very limited representation in Galicia to the areas with a greater Mediterranean influence (SE Galician territory). The presence of other *Quercus* species (*Quercus faginea* Lam. or *Quercus petraea* (Matt.) Liebl.) is limited to small areas. Ourense, located in the confluence of Eurosiberian and Mediterranean regions (Izco 1994; Rivas-Martínez 1987), and Vigo are the cities with a higher *Quercus* species diversity. Moreover, ornamental *Quercus* species are used in the four cities (being *Q. rubra* L. and *Quercus palustris* Muenchn the most common) which also influence the atmospheric *Quercus* pollen trapped.

Aerobiological monitoring

Volumetric LANZONI VPPS 2000 Hirst design spore traps (Hirst 1952) were used in all areas. They were located at a similar height from the ground (15–20 m) in the biology faculty in Santiago, the sciences faculty in Ourense, the city hall building in Vigo and the fire fighters building in Lugo. The study period began in 1993 in Santiago and Ourense, 1995 in Vigo and 1999 in Lugo. In all cases, the last studied year was 2012.

Aerobiological methodology was based in the model proposed by the Spanish Aerobiological Network (Galán et al. 2007). The *Quercus* pollen content in the air was expressed as daily mean pollen concentrations per cubic metre of air (pollen grains/m³).

The period covered by the airborne pollen season (APS) for each study year and sampling station was determined with reference to criteria suggested by Andersen (1991) and Jato et al. (2002). According to the first (95 % method), the start day is considered when the accumulated sum of pollen reached 2.5 % of the total annual (APS onset) and the final date (APS end) the day when the 97.5 % is reached. The APS

Table 1 Biogeographical and climatic characteristics of the study sites (meteorological data referred to the 1977–2007 period)

Biogeographical Characterization					Altitude (m a.s.l.)
Ourense	Eurosiberian reg. Cántabro-Atlántica Prov. Galaico-Portugués Sector. Lucense Subsector				454
Lugo	Eurosiberian reg. Cántabro-Atlántica Prov. Galaico-Portugués Sector. Miñense Subsector				50
Vigo	Eurosiberian reg. Cántabro-Atlántica Prov. Galaico-Portugués Sector. Compostelano Subsector				270
Santiago	Mediterranean reg. Carpetano-Iberico-Leonesa Prov. Orensano-Sanabriense Sector. Orensano Subsector				120
Climatic characterization					
	Average annual temperature	Annual maximum temperature average	Annual minimum temperature average	Annual total rainfall average sum	Geographical location
Ourense	14.8	20.9	8.9	873	42° 20' N–7° 52' W
Lugo	11.5	16.8	6.3	1,084	43° 0' N–7° 53' W
Vigo	14.1	18.0	10.2	1,686	42° 14' N–8° 44' W
Santiago	13.4	18.0	8.8	1,913	42° 53' N–8° 32' W

is therefore considered as the period during which the 95 % of the total annual is recorded. According to the criterion defined by Jato et al. (2002), the onset and ending dates were considered respectively when the prime or last pollen grains were registered on a continuous basis and no more than 2 consecutive days without pollen was detected. Andersen's method is more restrictive, in that it eliminates the long low-value tails at the start and end of the APS, thus favouring more accurate statistical analysis. The Jato et al. method, however, provides a more reliable interpretation of pollen-curve behaviour as a function of environmental factors (particularly weather-related factors at the beginning of pollen dispersal).

The date in which the highest annual daily mean pollen concentration was recorded was established as the peak date. The running 3-day average of the daily mean pollen concentration was used to obtain the data set pollen concentration average. The pollen index—PI—(the total annual *Quercus* pollen amount recorded) was considered as the pollen season severity.

The onset and end dates of the APS, the APS length, peak and pollen index, were the parameters applied to characterize the *Quercus* APS.

Thermal requirements

Chilling requirements

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

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

where CU=number of chilling units during the period,

$$B = 24D(T_{\text{threshold}} - T_{\min}) / (T_{\max} - T_{\min}).$$

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

Threshold temperatures from 3 °C to 12 °C in steps of 0.5 °C were also considered. Even these temperatures can be considered as high for chill calculate; the different climatic conditions of the four cities make convenient to test such temperature values.

Various criteria have been used to determine the period during which chilling accumulation takes place: (A) fixed start date for all years (1 November) and a range of completion dates (from 15 December to 31 January, increasing the calculation by 1 in 1 day); (B) fixed start date (1 November) and fixed end date (15 January) for all years; (C) Variable start and ending dates depending on threshold temperatures: (C.1)

taking into account the whole period from the first day when mean temperature fell below the threshold temperature selected (3–12 °C) to the day of the inflexion point registered by the minimum temperatures line when it was statistically transformed into a polynomial second-degree trend (generally occurred in the first half of January); (C.2) considering for the C.1 period, only those days on which weather conditions enabled no physiological activity in the tree, i.e. only days on which daily maximum temperature was equal to or lower than the threshold temperature (Montero and González 1983).

Heat requirements

According the results obtained in the previous studies conducted in Galicia (Jato et al. 2000; Rodríguez-Rajo et al. 2003, 2004, 2005), the following methods have been considered to calculate the heat accumulation (heat units=HU):

1. $HU = \sum T_{\max}$ method that used the sum of the daily maximum temperature.
2. $HU = \sum T_h$ method that used the difference between the daily maximum temperature and threshold temperature.

Temperature thresholds ranged from 6 to 8 °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: from the 16th of December, 1st and 16th of January and 1st of February. (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 of heat accumulation.

Weather-related parameters

The meteorological data used (daily mean, maximum and minimum temperature and rainfall) were provided 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 94 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 rainfall and number of rainy days from January to December.
- Seasonal averages for the above mentioned parameters.

Statistical treatment

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

In order to check the possible effect that the different number of years of the data set considered for each city could exert in the statistical results, a previous study was conducted by means of the comparison of the correlations and trends calculated considering both, the total period at the various sites and the same annual period (1999–2012) for all cities. Trend analyses carried out based on the same annual period at all sites showed only small differences from trend analyses using the total period at the various sites (except from Vigo, where start and end date trends of APS were insignificant when using the shorter period).

Results

Atmospheric pollen season (APS) characterization

The *Quercus* airborne pollen season was characterized at all four sampling stations (Table 2). Analysis of average daily mean pollen counts over the study period showed that the *Quercus* APS started in Vigo during the first fortnight in March, and began 9, 12 and 27 days later in Ourense, Santiago and Lugo, respectively (Table 2). The APS end occurs during the last days of May or the beginning of June when the Andersen method was used. It delays until the second fortnight of June in Ourense, Lugo and Vigo or the end of May in Santiago when the Jato et al. method was considered. The standard variations in the APS start and end dates during the years of study were slightly smaller using the Andersen method in almost all cases (even smaller for end date than for start date). Between cities, the greatest interannual variability was observed in Santiago. The length of the APS at the four sites was shorter considering the Andersen method (mainly for Lugo and Santiago with 54 and 61 days, respectively). APS length was similar in Ourense and Vigo using both methods, the longest season (107 days) being recorded in Ourense using the Jato method (Table 2).

The highest PI and the highest maximum daily mean pollen counts were recorded in Ourense. The lowest figures for both were recorded for Santiago. The *Quercus* PI ranged from 5 to 15 % of total annual pollen counts in Santiago and Ourense, respectively. On average, the highest monthly *Quercus* pollen counts were observed in April in Ourense (49.2 % of the *Quercus* PI), Vigo (43.3 %) and Santiago (59.9 %), whilst in Lugo, the highest mean monthly counts were recorded in May (51.8 %).

Average daily mean pollen counts for the study period as a whole were used to compare pollen-curve behaviour for the four cities (Fig. 2). The variations between the different curves obtained for each area according the two methods used to limit the APS can be checked by means of the kurtosis and skewness values (Table 3). The curves representing the APS according the Jato et al. method showed negative values of kurtosis with a more flat profile than the curves representing the APS according to the Andersen method. The peak recorded in Ourense at the beginning of the APS prompted the highest positive kurtosis value and a skewness value greater than 1, signifying the asymmetry of the curve. In all cases, the skewness shows positive values indicating the long tails of the curves, which it is more pronounced at the right. Important differences between years were observed in the APS pollen curves, especially between the rainy years and years with low annual total rainfall (during these years, successive peaks representing the different flowering periods of the various species and populations can be observed). However, persistent heavy rainfall over relatively long periods in certain years strongly influenced *Quercus* pollen emission (Fig. 3).

Thermal requirement

Chilling units

The onset date as calculated by the Andersen method was used to limit the chilling period, since this method recorded the lowest coefficient of standard deviation in all four cities.

Taking into account the lowest standard deviation coefficients obtained in each city (Table 4), 813 chilling units were accumulated in Ourense, 1,108 chilling units in Lugo, 664 chilling units in Vigo and 684 chilling units in Santiago. 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) (A method), the lowest standard coefficients for each city were obtained when the ending dates were considered during the second fortnight of December (in Ourense, Vigo and Santiago) or at the first fortnight of January (in Lugo). In every case, the highest chilling requirements were accumulated in Lugo and the lowest in Vigo.

Heat requirement

The first day of the APS or the peak date were considered as the end date of the heat accumulation. In Ourense and Santiago, the lowest standard deviation coefficient was obtained using the sum of daily maximum temperatures after deducting the threshold temperature. In order to compare the heat requirements between cities, the values obtained with the sum of daily maximum temperature were taking into account (Table 5). When the 16th of December was considered as

Table 2 *Quercus* APS onset/ending dates, length, peak and annual pollen index. APS calculated according to Andersen (1991) and Jato et al. (2002) methods

		<i>Quercus</i> APS (Andersen 1991)			
		Ourense	Lugo	Vigo	Santiago
Onset (date)	Average	24-Mar	11-Apr	15-Mar	27-Mar
	St. Dv.	8.64	9.52	8.47	11.16
	Minimum	11-Mar	31-Mar	28-Feb	11-Mar
	Maximum	12-Mar	27-Apr	3-Apr	19-Apr
End (date)	Average	6-Jun	3-Jun	1-Jun	26-May
	St. Dv.	9.02	6.88	12.98	16.12
	Minimum	20-May	22-May	1-May	18-Apr
	Maximum	19-Jun	17-Jun	30-Jun	19-Jun
Length (days)	Average	75	54	79	61
	St. Dv.	12.40	9.43	15.34	14.20
	Minimum	53 (2,011)	33 (2006)	57 (1995 and 1997)	36 (2000)
	Maximum	96 (1998)	66 (2009)	122 (2008)	88 (1993)
		<i>Quercus</i> APS (Jato et al. 2002)			
		Ourense	Lugo	Vigo	Santiago
Onset (date)	Average	12-Mar	4-Apr	7-Mar	22-Mar
	St. Dv.	7.51	9.84	9.05	12.79
	Minimum	1-Mar	22-Mar	13-Feb	28-Feb
	Maximum	28-Mar	22-Apr	21-Mar	17-Apr
End (date)	Average	26-Jun	16-Jun	19-Jun	30-May
	St. Dv.	13.92	7.68	12.59	16.70
	Minimum	6-Jun	1-Jun	30-May	18-Apr
	Maximum	28-Jul	26-Jun	26-Jul	24-Jun
Length (days)	Average	107	74	105	70
	St. Dv.	16.03	10.62	17.47	15.89
	Minimum	86 (2005)	52 (2006)	81 (2011)	39 (2000)
	Maximum	146 (2008)	92 (2002)	155 (2008)	100 (2004)
Peak (pollen/m ³ and date)	Average	335	196	221	91
	St. Dv.	307	90	131	72
	Minimum	77 (3/21/1998)	63 (5/28/2004)	58 (4/1/2001)	18 (3/19 and 3/28/1998)
	Maximum	1,212 (3/21/2009)	390 (4/24/2002)	507 (4/18/2011)	333 (4/12/1995)
Pollen index (annual total pollen and year)	Average	4,645	1,646	2,516	828
	St. Dv.	3,786	580	1,719	72
	Minimum	1,344 (1998)	580 (2010)	629 (2001)	180 (1996)
	Maximum	14,484 (2009)	2,805 (2002)	6,345 (2011)	2,152 (1995)

the start date of heat accumulation, lower standard deviation coefficients were registered than the obtained considering as the start date the following day when the chill requirement has been completed. Ourense and Lugo were the cities with the highest heat requirements.

Trends

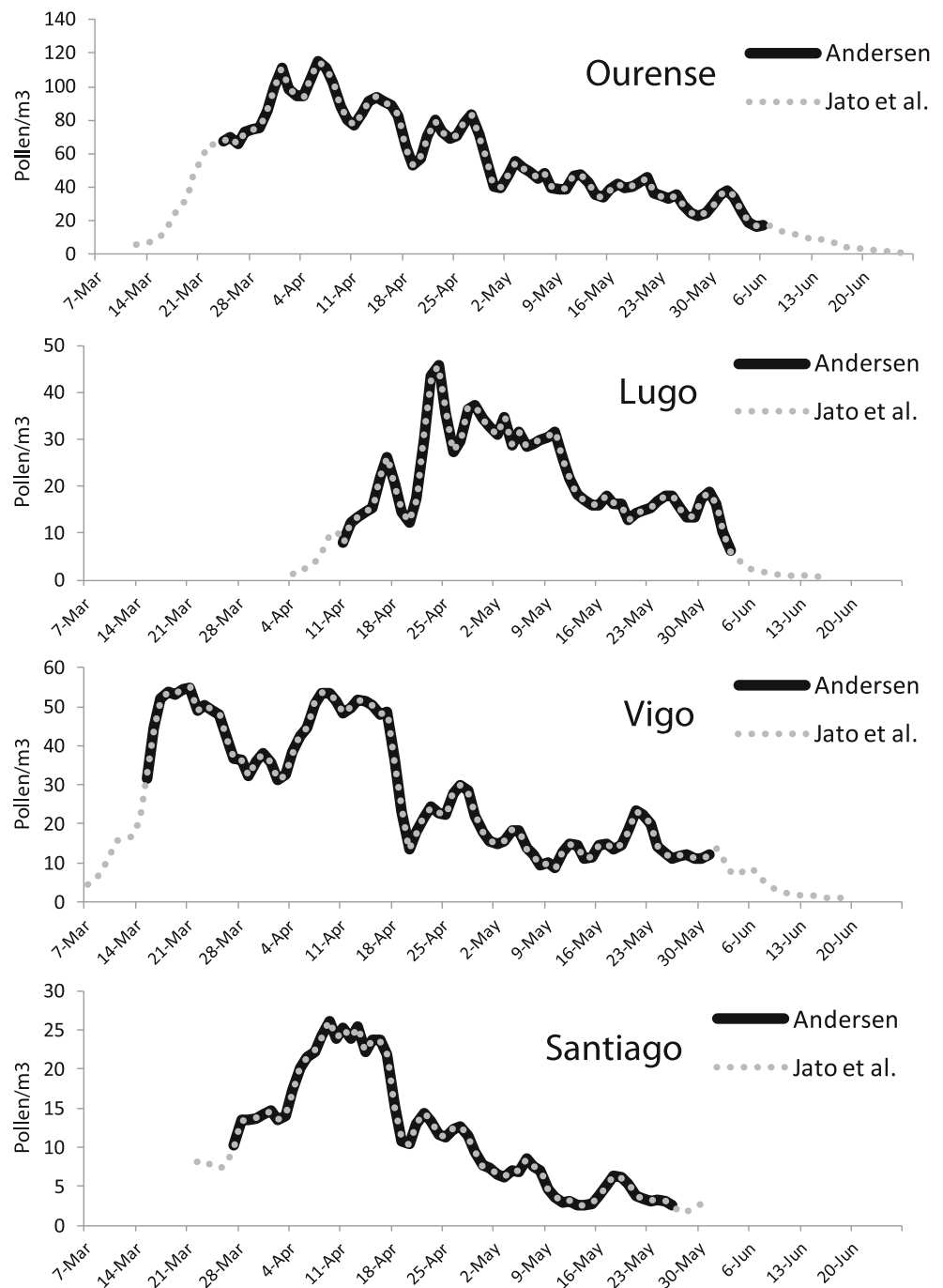
In trend analyses used in this chapter carried out by means, the total period at the various sites showed only small differences from trend analyses using the same annual period (1999–2012) at all sites, except from Vigo, where start

and end date trends of APS were insignificant when using the shorter period.

APS trends

A significant trend towards a later *Quercus* APS start date was observed at Ourense, Lugo and Vigo. A significant trend to a delay of the APS end date was observed in Vigo (slope=0.714; adj R^2 =0.783; p <0.01) and Lugo (slope=0.675; adj R^2 =0.772; p <0.01). Similar tendencies were observed for the peak date (slope=0.695; adj R^2 =0.677; p <0.01 in Vigo, slope=0.598; adj R^2 =0.577;

Fig. 2 All years 3-day running mean *Quercus* pollen average according to Andersen (1991) and Jato et al. (2002) methods in the four cities. X-axis: APS day number represented by the mean date



$p < 0.01$ in Lugo and slope=0.472; adj $R^2=0.312$; $p < 0.01$ in Ourense) (Table 6). No significant trend was detected in the four cities for the length of pollen season.

A significant trend to an increase of the annual total pollen and the annual maximum daily mean pollen concentrations of *Quercus* was observed in Ourense (slope=489.1; adj $R^2=0.561$; $p < 0.01$ and slope=37.854; adj $R^2=0.505$; $p < 0.01$ respectively). A similar but not significant tendency was detected in Vigo. Otherwise, a non-significant trend towards lower annual *Quercus* annual pollen indices was noted

in Santiago and Lugo, whilst a trend towards lower maximum daily mean pollen counts was observed in Santiago.

Thermal requirements trends

Apart from a slight trend towards increased accumulated chilling periods in all four cities, no significant trends were noted (Table 6). A trend to an increase of the heat requirements needed for the *Quercus* APS onset in the four cities was noted. This tendency was significant for Ourense and Vigo

Table 3 Kurtosis and skewness values of the APS curves. APS calculated according to Andersen (1991) and Jato et al. (2002) methods

Method	City	Valid <i>N</i>	Skewness	Std. Err. skewness	Kurtosis	Std. Err. kurtosis
Jato et al. (2002)	Ourense	107	0.577	0.234	−0.757	0.463
	Lugo	74	0.876	0.279	−0.236	0.552
	Vigo	105	0.502	0.236	−1.049	0.467
	Santiago	70	0.777	0.287	−0.637	0.566
Andersen (1991)	Ourense	75	1.451	0.277	1.619	0.548
	Lugo	54	0.446	0.325	−0.442	0.639
	Vigo	79	0.964	0.271	0.038	0.535
	Santiago	61	0.836	0.306	−0.159	0.604

considering as the first day of heat accumulation was on the 16th of December (slope=10.620 and 18.460, respectively; $p<0.05$ in both cities). Also, a significant trend was noted for Lugo and Ourense when the heat accumulation was calculated from the first day after the chilling period to the first APS day (slope=22.900; adj $R^2=0.236$, $p<0.05$ and slope=10.620; adj $R^2=0.093$, $p<0.05$, respectively). Finally, significant tendencies were achieved for Vigo and Santiago considering as the last day of accumulation was on the peak date (slope=46.490; adj $R^2=0.253$, $p<0.05$ and slope=28.960; adj $R^2=0.287$, $p<0.01$, respectively).

Meteorological trends

A trend to an increase of the annual average of the maximum, minimum and mean temperatures were observed in Lugo, Ourense and Vigo (Table 6). However, only the trend was significant for minimum temperature in Vigo (slope=0.120; adj $R^2=0.179$, $p<0.05$). A significant trend to a decrease of the annual rainfall has been observed in all cities, being significant in Ourense and Lugo (slope=−16.19; adj $R^2=0.157$, $p<0.05$ and slope=−30.91; adj $R^2=0.269$, $p<0.05$).

To determine whether the trends detected were uniform throughout the year, seasonal and monthly weather data were also analyzed. A significant trend towards increasing minimum temperatures in spring was observed in Lugo (slope=0.117; adj $R^2=0.428$, $p<0.01$) and Vigo (slope=0.172; adj $R^2=0.301$, $p<0.05$), and a slight trend towards decreasing mean winter temperatures was recorded in Santiago (slope=−0.067; adj $R^2=0.219$, $p<0.05$). A decrease of rainfall in summer was detected in Lugo (slope=−11.984; adj $R^2=0.526$, $p<0.05$) and Santiago (slope=−7.854; adj $R^2=0.219$, $p<0.05$). A general significant increase in maximum September temperatures was detected in all four areas (Table 6); the trend being most marked in Vigo (slope=0.261; adj $R^2=0.443$; $p<0.01$). The same trend was also noted for the mean and minimum temperatures in Ourense and Vigo. Vigo was the only city where a significant tendency to an increase of the temperature in March (minimum temperature), May (maximum, minimum and mean

temperatures) and June (for minimum temperature) was detected. The months of May, July and September showed a significant trend to a rainfall decrease in Ourense, in May and September in Santiago, in September in Lugo and May for Vigo.

Discussion

Quercus is well-represented in Galicia by several spring-flowering species. The APS starts first in Vigo and Ourense (the most southern localities), occurring later in Santiago and particularly in Lugo. Airborne pollen counts provide useful information on the timing of flowering (Frenguelli et al. 1992; Chuine et al. 1998; Jato et al. 2002; Grewling et al. 2012). Pollen grains are released and dispersed during anthesis, and differences in biogeographical location, topography and the presence of nearby ornamental *Quercus* species lead to differences in airborne pollen counts between the four cities. According to a phenological survey carried out by Jato et al. (2002), which focused on the behaviour of *Quercus* species native to the Galicia region, the first species to flower is *Q. robur*, closely followed by *Q. ilex* and *Q. faginea*. *Q. pyrenaica* flowers at the end of the *Q. robur* flowering period. The last *Quercus* species to flower is *Q. suber* whose pollination period does not overlap with any flowering period of other *Quercus* species. According to the pollen production, the most important sources of *Quercus* pollen in the atmosphere of Galicia are the *Q. robur* and *Q. pyrenaica* species (Dacosta 2005).

The survey disclosed certain differences between the *Quercus* pollen curves obtained in the four cities studied. *Q. robur* was the major pollen source at the start of the APS, during the second fortnight in March (mainly in Vigo, Ourense and Santiago) or at the beginning of April (in Lugo). Although *Q. rubra* in general produces less pollen than *Q. robur* (Dacosta 2005), the widespread use of *Q. rubra* as an ornamental might account for the contribution of this species to the pollen curve at an early stage, especially in Santiago. In the more southerly cities (Vigo and Ourense),

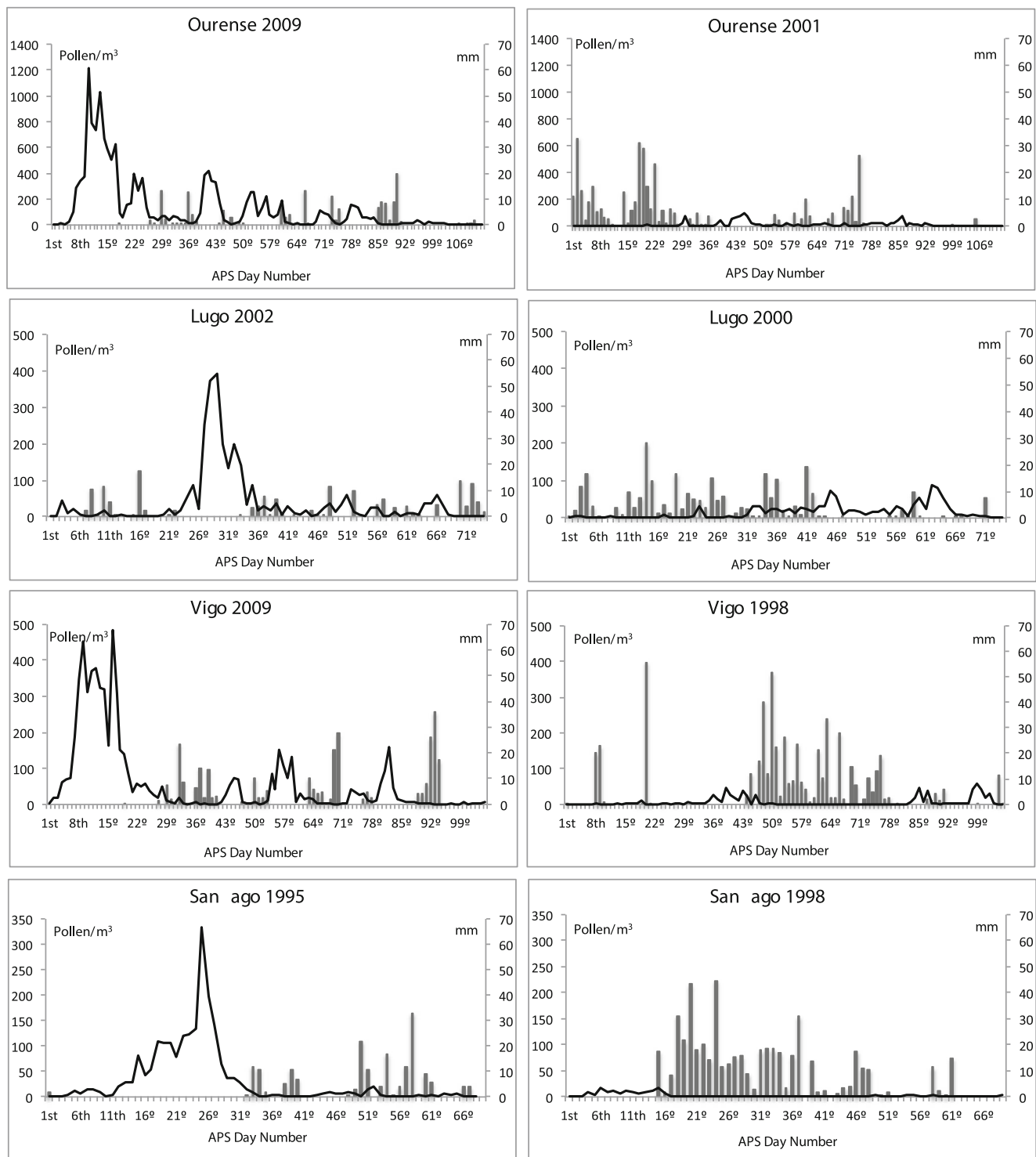


Fig. 3 Pollen curves representing the APS in selected rainy (right) and dry (left) years. APS according to Jato et al. (2002) method. Y-axis: daily mean pollen concentrations. X-axis: APS day number (line pollen, bar rainfall)

Q. ilex may also contribute considerably to the pollen curve (Jato et al. 2007). Abundant *Q. pyrenaica* prompted a second peak in April in these cities, and particularly in Ourense. Moreover, the pollen season is extended to the month of May and the first fortnight of June in the southerly cities due to the flowering of *Q. suber*. By contrast, the limited presence

of *Q. pyrenaica* in the more northerly cities led to a comparatively shorter pollen season. The contribution of other *Quercus* species was negligible, since they are relatively scarce in the region. The highest pollen counts were recorded in Ourense, possibly due to the greater species diversity and to a greater abundance of *Q. pyrenaica* (as *Q. robur* and

Table 4 Chilling units achieved by the APS onset and *Quercus* peak dates calculated with different methods (see ‘Chilling requirements’) and starting dates. APS onset calculated according to the Andersen (1991) method

	Method	Period	Th °C	CU	St. Dv. Cf.
Ourense	A	11/1–12/15	7.2	755	14.4
Vigo		11/1–12/19	7.2	664	4.6
Santiago		11/1–12/15	7.2	684	7.8
Lugo		11/1–1/13	7.2	1,053	7.1
Ourense	B	11/1–1/15	7.2	925	18.7
Vigo		11/1–1/15	7.2	635	23.4
Santiago		11/1–1/15	7.2	772	14.6
Lugo		11/1–1/15	7.2	1,045	8.3
Ourense	C.1	12/6–1/2	4.5	783	8.4
Vigo		12/1–12/27	7.5	734	9.6
Santiago		12/2–1/1	6.5	761	8.8
Lugo		11/10–1/2	7.5	1,108	6.5
Ourense	C.2	11/15–1/2	8.5	813	5.8
Vigo		11/22–12/27	10.0	780	6.4
Santiago		11/1–1/1	9.5	820	10.7
Lugo		11/10–1/2	7.5	919	9.1

CU chilling unit, Th °C threshold temperature, St. Dv. Cf. standard deviation coefficient

Q. pyrenaica were considered high pollen production species). Moreover, local topography in Ourense may play a key role by providing conditions suitable for medium-distance pollen transportation (Jato et al. 2007); the location of different *Quercus* species at different altitudes and in different directions with regard to the city facilitates transport of the pollen over a wide area (10–30 km away from the trap).

Table 5 Heat accumulation (see ‘Heat requirements’) following the $\Sigma T_{\max}$ method from 16th December and the end of chilling date to the *Quercus* APS onset (calculated according Andersen (1991) method) and from the 16th December to the peak date

	Period	HU	St. Dv. Cf.
Ourense	12/16—APS onset	1,512	7.64
Vigo	12/16—APS onset	1,280	14.05
Santiago	12/16—APS onset	1,435	9.64
Lugo	12/16—APS onset	1,487	9.12
Ourense	End chilling—APS onset	1,313	14.70
Vigo	End chilling—APS onset	1,148	25.04
Santiago	End chilling—APS onset	1,245	13.46
Lugo	End chilling—APS onset	1,322	13.36
Ourense	12/16—peak date	1,827	10.40
Vigo	12/16—peak date	1,849	24.63
Santiago	12/16—peak date	1,771	16.99
Lugo	12/16—peak date	1,907	13.35

HU heat unit, St. Dv. Cf. standard deviation coefficient

The differences observed in the APS onset dates between the four areas are a consequence of the climatic differences, being in all cities; *Q. robur* and *Q. rubra* were the sources of the earliest *Quercus* pollen captured.

The choice of method for limiting the pollen season may vary depending on a number of factors, including the site, pollen type and amount of pollen in the air, and is often dictated by the purposes of the study (Jato et al. 2006). In most cases, the aim is to eliminate long low-value tails at the start and the end of the pollen season. This fact is especially important for the statistical analysis (Sánchez-Mesa et al. 2003). In this survey, differences between the curves obtained by means of the two methods (related to tails of low values at the beginning and the end of the APS) were pointed out by means of the kurtosis and skewness coefficients. The method proposed by Jato et al. (2002) provided a more useful overview of the whole *Quercus* APS since the Andersen (1991) method has the disadvantage that the elimination of tails from the APS involves days with daily mean pollen counts above 50 pollen grains/m³ (even 80 pollen grains/m³) in the cases of years with a high *Quercus* PI.

Airborne pollen counts are greatly affected by the weather conditions that influence pollen production, anther dehiscence, and pollen-grain release and dispersal. Meteorological factors also influence medium-long-distance transport and re-suspension from the ground, thus considerably modifying pollen curves. Rainfall patterns have a marked impact on airborne pollen dynamics (García-Mozo et al. 2006; Jato et al. 2007). During spring, rainy days are frequent in Galicia. Precipitation regimes varied as a function of the climate regime in the four study cities inducing a strong influence on the pollen curve behaviour. In this survey, the years with warm and sunny days without rainfall during the flowering period (as occurs during 1995, 1997, 2002 and 2009) facilitates the pollen liberation and its dispersion in the atmosphere. These years, the pollen curve better reflect the real flowering of the different *Quercus* species in Galicia. However, in years characterized by a rainy spring (such as 1998, 2000, 2001, 2004), pollen counts tended to be lower, and the successive flowering of different *Quercus* species was not clearly reflected in pollen curves. In years with a prolonged rainy period, anther opening was hindered, thus inhibiting pollen release and dispersal. This process is especially important in the city of Vigo where the rainfall during the APS is higher than in the others localities. In this case, a significant and negative correlation was achieved between the total annual *Quercus* pollen amount and the rainfall (adj $R^2=0.529$, $p<0.05$). The same tendencies were achieved in the other three cities whilst the correlations were not significant.

Chilling accumulation is necessary in order to break dormancy. Once the chilling is completed, the phenological phase of flowering is determined by the temperatures recorded during the previous 25 to 55 days (Jato et al. 2004; Rodríguez-

Table 6 *Quercus* APS, chill and heat requirements and meteorological trends observed

	Ourense			Lugo			Vigo			Santiago		
	Slope	Adj. R^2	p	Slope	Adj. R^2	p	Slope	Adj. R^2	p	Slope	Adj. R^2	p
<i>Quercus</i> APS trends												
<i>Quercus</i> APS onset (95 %)	0.650	0.294	0.008	0.404	0.287	0.028	0.635	0.649	0.000	−0.041	–	0.898
<i>Quercus</i> APS ending date (95 %)	0.192	0.021	0.249	0.675	0.772	0.000	0.714	0.783	0.000	−0.027	–	0.938
<i>Quercus</i> peak value date	0.472	0.312	0.006	0.598	0.577	0.001	0.695	0.677	0.000	0.289	–	0.435
<i>Quercus</i> APS length (95 %)	−0.871	0.127	0.068	−0.659	0.009	0.310	0.249	–	0.733	0.249	–	0.664
Annual <i>Quercus</i> pollen	489.100	0.561	0.000	−54.840	0.086	0.162	93.092	0.026	0.245	−8.065	–	0.749
<i>Quercus</i> peak value	37.854	0.505	0.000	1.349	–	0.831	7.021	0.024	0.251	−3.134	0.015	0.271
Chill and heat requirements trends												
Chill units	6.187	–	0.449	5.969	0.006	0.318	2.650	–	0.706	7.202	0.061	0.167
Sum Tmax (16/12 to onset APS)	10.620	0.257	0.013	16.460	0.196	0.064	18.460	0.257	0.018	8.442	0.082	0.118
Sum Tmax (end chill to onset APS)	10.620	0.093	0.013	22.900	0.236	0.045	16.635	0.039	0.212	4.639	–	0.490
Sum Tmax (16/12 to peak date)	−2.659	0.002	0.321	4.369	–	0.807	46.490	0.253	0.019	28.960	0.287	0.009
Annual, seasonal and monthly weather-related parameters trends												
Annual maximum temperature average	0.022	–	0.359	0.055	0.037	0.245	0.099	0.110	0.097	−0.007	–	0.76
Annual minimum temperature average	0.008	–	0.733	0.050	0.103	0.140	0.120	0.179	0.045	−0.027	0.105	0.09
Annual medium temperature average	0.003	–	0.894	0.026	0.003	0.327	0.066	0.029	0.236	0.0005	–	0.988
Annual rainfall	−16.190	0.157	0.047	−30.910	0.269	0.033	−33.250	0.104	0.104	−27.530	0.086	0.112
AMJ—seasonal minimum temperature	0.055	0.118	0.076	0.117	0.428	0.007	0.172	0.301	0.011	0.012	–	0.560
JFM—mean temperature	−0.039	–	0.244	−0.012	–	0.869	0.123	0.165	0.053	−0.067	0.219	0.022
JAS—rainfall	−6.773	0.134	0.066	−11.984	0.526	0.002	−6.710	0.099	0.110	−7.854	0.217	0.023
March minimum temperature	0.007	–	0.899	−0.062	–	0.554	−0.141	0.247	0.021	0.035	–	0.340
May maximum temperature	0.105	0.110	0.084	0.088	0.032	0.255	0.157	0.264	0.017	0.059	0.149	0.271
May minimum temperature	0.035	–	0.401	0.133	0.181	0.073	0.174	0.325	0.008	−0.001	–	0.962
May mean temperature	0.056	0.060	0.153	0.065	0.043	0.231	0.154	0.296	0.012	0.015	–	0.638
June minimum temperature	0.061	0.085	0.113	0.072	0.035	0.248	0.171	0.330	0.007	0.010	–	0.726
September maximum temperature	0.261	0.443	0.001	0.187	0.333	0.018	0.241	0.334	0.007	0.227	0.313	0.006
September minimum temperature	0.094	0.216	0.023	0.090	0.002	0.331	0.194	0.291	0.012	0.050	0.064	0.147
September medium temperature	0.161	0.450	0.001	0.077	0.039	0.240	0.154	0.199	0.036	–	–	–
October maximum temperature	0.121	0.066	0.144	0.268	0.336	0.014	0.147	0.072	0.147	0.056	–	0.386
May rainfall	−4.140	0.240	0.019	−1.593	–	0.552	−5.462	0.251	0.019	−8.826	0.405	0.002
July rainfall	−7.156	0.157	0.047	−1.748	0.021	0.280	−0.906	–	0.511	0.555	–	0.753
September rainfall	−5.378	0.188	0.032	−7.413	0.360	0.014	−6.132	0.113	0.094	−7.329	0.255	0.014

Italic numbers indicate significant values. Analysis time period 1993–2012 in Santiago and Ourense, 1995–2012 in Vigo and 1999–2012 in Lugo

Rajo et al. 2006). Requirements with regard to chilling and heat accumulation differed depending on local climate conditions in the study cities: the lowest values for chilling accumulation were recorded in Vigo and the highest in Lugo. Colder winter conditions in the inland cities (Lugo and Ourense) favoured greater chilling accumulation, especially in Lugo, where the minimum temperatures recorded in January delayed heat accumulation and thus the onset of the APS. Other factors, including rainfall and biotic factors such as insect pests at the start of the season, can delay the onset of the APS, by reducing airborne pollen counts and tree pollen production (Jato et al. 2002). In our survey, the lowest heat requirements were registered in Vigo. Slight differences

between the other three areas were noted. In the same way, low differences were observed between the four cities in relation to the heat accumulated to the peak date. The slight variations in the chill accumulation were compared with that pointed out in previous works (Rodríguez-Rajo et al. 2003; Rodríguez-Rajo et al. 2005) could be related to the different number of the study period years included in the survey, as well as the different threshold temperature applied. In our study, trend analyses carried out based on the same annual period at all sites showed only small differences from trend analyses using the total period at the various sites (except from Vigo, where start and end date trends of APS were insignificant when using the shorter period). When we

compare the different methods and threshold temperatures used to calculate chilling and heat requirements, the lowest coefficient of standard deviation for chilling were obtained with threshold temperatures from 4.5 to 7.5 °C. Similar threshold temperatures for the calculation of chilling and heat requirements (ranging from 4.5 to 7.5 °C) have been reported in earlier studies of *Quercus* and other tree species in the same geographical area (Jato et al. 2000; Rodríguez-Rajo et al. 2003, 2004, 2005).

Changes in the vegetation have proved to be very sensitive indicators of climate change. Over recent decades, numerous surveys have been carried out in Europe in order to ascertain the influence of climate change on phenological events. Research on *Q. robur* shows that over the period 1959–1996, European spring events advanced by an average of 6.3 days, whereas autumn events were delayed by 4.5 days; the annual average growing season has therefore lengthened by 10.8 days since the 1960s (Menzel et al. 2006). Changes in temperature patterns over the preceding months have led to a 2.5-day advance in spring/summer phases, variations being more marked in Spain than in many other European countries (Menzel et al. 2006). Similar results were obtained in leaf unfolding and flowering from the beginning of the 1970s in Spain, showing an advance, more acute in anemophilous than entomophilous species (Gordo and Sanz 2009). The results obtained here reveal a clear tendency towards delayed onset of *Quercus* APS and a similar delay in peak pollen dates in three of the four study cities, together with delayed ending of the APS in Lugo and Vigo. However, since trends with regard to pollen season start and end dates differed between cities, overall trends in pollen season length for the study area as a whole were not significant. The possible influence of climate change on the APS and on pollen production has been studied in various countries and species; this research has highlighted the value of aerobiological analysis as a tool for detecting possible changes in vegetation. Changes in the APS onset have been noted, for example: birch, hazel, chestnut, alder, nettle and oak (Emberlin et al. 1997; Emberlin et al. 2002; Frei and Gassner 2008; Gordo and Sanz 2009). An earlier oak flowering associated with increased temperatures in the pre-flowering periods has been noted in Spain (García-Mozo et al. 2006; Shea et al. 2008). Trends to a longer pollen season, greater exposure and increased disease burden for late summer weeds (such as mugwort and ragweed) have already been demonstrated (Stach et al. 2007; Breton et al. 2006). In phenological studies conducted in Galicia, a significant advance in the flowering start date was signalled for chestnut (Perez Muñuzuri et al. 2009). Earlier research suggests that there is no uniform significant trend for different tree species and different cities in Galicia, although a non-significant trend towards delayed APS onset has been reported for *Alnus* and *Betula*, together with a slight advance for *Castanea* (Rodríguez-Rajo et al. 2010; Jato et al. 2012). Differences

with regard to a possible trend towards delayed *Quercus* APS onset within Galicia may reflect differences in the meteorological trends detected. A non-uniform heating through the year in Galicia was observed, which is more important in the southwest: an increase in May and June temperatures was recorded, but there was no significant trend towards rising winter temperatures. Therefore, changes in chilling and heat accumulation could influence the start of vegetative activity after dormancy, prompting a delay in APS onset.

Pollen production and distribution of allergen-producing species can be also affected by climatic change. Weber (2002) has suggested that one of the implications of the increased pollen production associated with the increased CO₂ concentration could induce a more efficient wind pollination and a greater propagation of the species (Beggs 2004). Using aerobiological data, pollen increases over the past 30 years has been proved in hazel, birch and grass counts in Switzerland and Denmark (Spieksma et al. 1995; Frei 1998). Statistically significant increases have been reported in total seasonal pollen counts for various taxa including *Quercus*, associated with an increase in average winter temperatures (Levetin 2001). Increases in *Quercus* pollen counts have been predicted for a number of Spanish cities, especially in inland locations (García-Mozo et al. 2006). The present study observed a significant trend towards an increase in the *Quercus* pollen index in Ourense. The same tendency, but not significant, was detected in Vigo, whereas the opposite, but not significant trend, is showed in Lugo and Santiago. Although differences between cities are not easily explained, it should be noted that a similar significant trend towards increasing annual pollen indices has been reported for *Betula* in Ourense and for *Castanea* in both Ourense and Vigo (Jato et al. 2012). The weak trend to a decrease of the annual rainfall does not justify the changes detected for the pollen production. Biogeographic and climatic characteristics, as well as human action, can affect airborne pollen counts in each area (Galán et al. 2007). Inter-city differences may be linked to the more marked evidence of climate change detected in the south of Galicia compared to more northerly cities. Moreover, examination of transitional zones reveals that vegetation responses can occur quickly and might be detected within a decade of climate change (Peteet 2000). The transitional location, particularly of Ourense, between the Eurosiberian and Mediterranean biogeographical areas might favour a more acute response to climate change in the south of the region.

Conclusions

The length and intensity of the *Quercus* APS was greatest in Ourense and lowest in Santiago. Differences in *Quercus* species diversity, in air temperatures recorded during the period

prior to the APS and in thermal requirements account for the variations observed between the APS in the four study cities.

Differences in APS trends between cities reflected differences in weather-related trends. A significant trend towards rising *Quercus* pollen indices and higher maximum daily mean pollen counts was observed in Ourense, linked to the more marked temperature increase across southern Galicia.

Although no significant tendency towards increased winter temperatures was detected, changes in chilling and heat-accumulation requirements influence the start of vegetative activity after dormancy, prompting a delay in APS onset.

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