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Phenological behaviour of *Quercus* in Ourense (NW Spain) and its relationship with the atmospheric pollen season

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Abstract Knowing the beginning of the pollen season is of particular importance to people allergic to a given pollen. Phenological studies in combination with aerobiological studies enable us to observe the relationship between the reproductive phenology of the species and their airborne pollen curves. They also enable us to study the relationship between the release of pollen from anthers and its presence in the atmosphere. The objectives of this study are (1) to determine chill and heat requirements for different phenological phases in the *Quercus* species present in Ourense (Spain), (2) to verify the synchronization between the flowering event and the presence of *Quercus* pollen in the air, (3) to select the best criterion for determining the onset of the pollen season according to phenological data, and finally, (4) to obtain models enabling us to determine the onset of the pollen season. Pollen monitoring was carried out from 1993 to 2001, by means of a 7-day Lanzoni VPPS pollen trap and, in order to characterize the floral phenophase of different *Quercus* species, a sampling method was applied from February to June 2001. We observed that climatic and biotic factors can produce a delay in the onset of the pollen season. Temperatures above 8 °C and between 6 °C to 8 °C are good threshold temperatures for estimating chill and heat requirements respectively. Medium-distance transportation of *Quercus robur* and *Quercus pyrenaica* pollen from areas 10–30 km away influences the concentrations of *Quercus* pollen registered in Ourense.

Keywords Phenology · *Quercus* · Aerobiology · Airborne pollen · Chilling requirements · Heat requirements · Spain · Ourense · *Neuropterus*

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Introduction

One of the most important objectives of aerobiological studies is to find prediction models enabling us to ascertain both the onset and severity of a pollen season. Knowing the beginning of the pollen season is of particular importance to people allergic to a given pollen, as this would enable them to begin an anti-allergic treatment several days before the beginning of pollination, thereby optimising its effectiveness.

Atmospheric pollen is greatly influenced by meteorological conditions. Before flowering, temperature and sunshine are the primary factors influencing the growth and development of plants and pollen production. During the flowering period, secondary factors, such as sunshine, rainfall and relative humidity determine the opening of anthers and the release of pollen grains. Once the pollen is dispersed, the wind (tertiary factor) will scatter the pollen in the atmosphere (Laaidi 2001).

The developmental process in the bud that releases dormancy and thus triggers the onset of growth in temperate-zone trees is mainly regulated by temperature. This regulation is such that a period with chilling temperatures, depending on the species and geographical areas, followed by a period with forcing temperatures (also variable), induces budburst. These periods differ with the species and geographical area. Rest is defined as the period in which buds remain dormant because of growth-arresting physiological conditions in the bud itself. The growth-arresting conditions are eliminated when buds are exposed to chilling temperatures for a certain period. The subsequent stage of dormancy is called quiescence. Quiescence is defined as the period in which the buds remain dormant because of unfavourable environmental conditions. Bud burst takes place when the buds are exposed to forcing temperatures for a prolonged period (Kramer 1994).

Different indexes can be referenced to determine chill and heat requirements. Chilling units or chilling hours are frequently used to define chill requirements (Aron 1983). Heat units (degree-hours or degree-days) are a

measure of the duration of one phenological stage and are often used to define the phenological development of plants. One degree-hour is observed when the air temperature is 1 degree above a lower threshold temperature for 1 h. Development rates are assumed to be insignificant when the air temperature is below the threshold. If the temperature is more than 1 degree above the threshold temperature for 1 h, then the number of degree-hours equals the observed air temperature minus the threshold temperature. Degree-days are calculated as the total degree-hours for a day divided by 24 (Snyder et al. 1999). Other indexes such as accumulated daily maximum temperatures are also employed, especially when aerobiological data are used (Garcia-Mozo et al. 2000; Galán et al. 2001).

Many phenological studies have been conducted to determine the chill and heat requirements in different species and geographical areas (Kramer 1994; Snyder et al. 1999; Spano et al. 1999; Wielgolaski 1999). Phenological events can be detected by observing plants (opening of buds, leaf unfolding, flowering and so on) and they enable us to determine the precise date that the event occurred and to calculate the heat requirements for transition between phenological stages. These requirements usually vary according to the species and geographical area. It should be further noted that different plant populations in any given area can have various heat requirements.

During the last few years studies using aerobiological data to determine chill and heat requirements have been conducted (Frenguelli and Bricchi 1998; Garcia-Mozo et al. 2000; Jato et al. 2000; Rodríguez-Rajo et al. 2000; Galán et al. 2001; Fornaciari et al. 2000; Laaidi 2001). Assuming that atmospheric pollen is a consequence of the flowering of plants, then aerobiological data can be used to determine heat requirements and therefore the onset of the pollen season. However, there are some questionable factors in these studies, as explained below. The onset of the pollen season can be defined by different criteria (Mullenders et al. 1972; Nilsson and Persson 1981; Torben 1991; Galán et al. 1995; Garcia-Mozo et al. 1999), so we need to determine which corresponds best to the phenological data. The beginning of the pollen season can be altered by long- and medium-distance pollen transportation, so we need to determine the synchronization between flowering and atmospheric pollen. Environmental factors, such as rainfall and biotic factors at the beginning of flowering, can produce a reduction in atmospheric pollen or in pollen production and therefore delay the onset of the pollen season. Finally, different species can be included in the same pollen type, so we need to know which one flowers first.

Phenological studies in combination with aerobiological studies enable us to observe the relationship between the reproductive phenology of species and their airborne pollen curves. It also allows us to study the relationship between the release of pollen from anthers and its presence in the atmosphere (Latorre and Bianchi 1998; Fornaciari et al. 2000). The objectives of this study are

(1) to determine chill and heat requirements for different phenological phases in the *Quercus* species present in our territory, (2) to verify the synchronization between the flowering event and the presence of *Quercus* pollen in the air and to determine the importance of each species of *Quercus* in the pollen curve, (3) to select the best criterion for determining the onset of the pollen season according to phenological data, and (4) to obtain models enabling us to determine the onset of the pollen season.

Materials and methods

The study was conducted in the city of Ourense, located in north-west Spain (42°0'N and 7°51'W) (Fig. 1). Its climate is oceanic with a strong Mediterranean influence. The mean annual temperature is 14 °C (the maximum average temperature is 18.9 °C and 9.2 °C is the minimum average temperature). Annual rainfall reaches 772 mm, with a very irregular distribution throughout the year, only 29 mm being collected during the summer months. The city occupies a depression (130 m above sea level) produced by the action of the rivers crossing through the area.

From a biogeographical point of view, Ourense is located in the confluence of Eurosiberian and Mediterranean regions (Izco 1987; Peinado-Lorca et al. 1987). Different types of *Quercus* forests are the predominant vegetation around the city. *Q. pyrenaica* and *Q. robur* are the most frequent oaks. *Q. suber* is located in the city's lowest areas. *Q. ilex* subsp. *ballota* has a very limited presence and only occupies a small area close to the city. Finally, *Q. faginea* is very rare and is only represented by a small number of trees intermingled with *Q. robur*, *Q. suber* and *Q. ilex* in one of the areas studied.

All of the meteorological data of the city of Ourense used in this study were obtained from the National Meteorological Institute.

Aerobiological survey

Pollen monitoring was carried out from 1993 to 2001 by means of a 7-day Lanzoni VPPS 2000 continuous volumetric pollen trap, located on the roof of the Science Faculty. The methodology recommended by the Spanish Aerobiological Network was used (Domínguez et al. 1992). The onset of the pollen season was defined according to three different criteria: when 2.5% (Torben 1991) and 5% (Nilsson and Persson 1981) of the annual total pollen was reached, and when the prime pollen grains were registered on a continuous basis and no more than 2 consecutive days without pollen were detected.

Phenological survey

A sampling method was applied from 1 February to the end of June 2001 in order to characterize the floral phenophase. Two areas located near the spore trap (2 km), with different orientations relative to the sun, were chosen. Five species of *Quercus* in the first area – Santomé – *Q. robur*, *Q. pyrenaica*, *Q. suber*, *Q. ilex* subsp. *ballota* and *Q. faginea* and three in the second area – Piñor – (*Q. robur*, *Q. pyrenaica* and *Q. suber*) were studied. In each population a sample of three individual randomly chosen trees was used, and for each tree the date of bud break, leaf unfolding and the start and end of flowering were noted. Phenological observations in each population were carried out at least once a week, increasing to twice a week during flowering.

The bud-break date was considered to be the first day that the first leaf-stalk of the plant or of the specific branch was observed (Wielgolaski 1999). The leaf-unfolding date was noted when at two or three places in the tree a normal, unwrinkled, leaf surface was visible, but full size had not yet been attained (Bos 1893). The onset of flowering was considered to be the first day a male catkin



Fig. 1 Location of Ourense in Spain

distributed its pollen (Wielgolaski 1999). The end of flowering was when all the catkins were dried.

Determining the chill requirement

We used the model proposed by Aron (1983) to obtain the chill accumulation expressed as chilling hours (CH):

$$CH = 801 + 0.2523B + 7.574 \times 10^{-4}B^2 - 6.51 \times 10^{-10}B^4 - 11.44T_{\min} - 3.32T_{\max}$$

where $B = 24D [(7.2 - T_{\min})/(T_{\max} - T_{\min})]$, D is the number of days in the period, $T_{\max}$ and $T_{\min}$ are the mean maximum and minimum temperatures of the period respectively.

We used this model, selecting the period with two different criteria:

1. Taking into account the period from the day when the mean temperature decreased below the threshold temperature to the day (in the first half of January) when the minimum was recorded and a change in the temperature trend was detected, or
2. Selecting the days in which meteorological conditions did not enable any physiological activity in the tree (vegetative activity method). This happens when the daily maximum temperature is equal to or less than the threshold temperature (Montero and González 1983).

Determining the heat requirement

Several methods for determining the heat requirement were tested taking into account the period from the day after the chill period to the start of the pollen season. Data from 8 years (1993–2000) of aerobiological monitoring were used. Calculations were carried out by several methods:

1. Using the mean of the daily mean temperatures (T_i) during the period (Yang et al. 1995):

$$^{\circ}D = \sum (T_i - T_h)n$$

where T_i is the sum of the daily mean temperatures divided by the number of days in the period.

2. Degree-days ($^{\circ}D$) were calculated by taking into account daily mean temperatures (Ring et al. 1983):

$$^{\circ}D = \sum (T_{\text{med}} - T_h)$$

where T_{med} is the daily mean temperature. If $T_{\text{med}} < T_h$ then $^{\circ}D$ is equal to 0.

Table 1 Mean dates of some phenological events of deciduous (*Q. pyrenaica*, *Q. robur*, and *Q. faginea*) and perennial (*Q. ilex* subsp. *ballota* and *Q. suber*) species of *Quercus*, observed in 2001

Species	Bud break	Leaf unfolding	Start flowering	End flowering
<i>Q. robur</i>	27/2/01	15/3/01	21/3/01	14/4/01
<i>Q. pyrenaica</i>	22/3/01	5/4/01	10/4/01	6/5/01
<i>Q. faginea</i>	7/3/01	14/3/01	22/3/01	5/4/01
<i>Q. ilex</i>			26/3/01	10/4/01
<i>Q. suber</i>			15/5/01	8/6/01

3. We also applied the criterion of Zalom et al. (1983), assuming that if $T_h \geq T_{\max}$, then $^{\circ}D = 0$, if $T_{\min} < T_h < T_{\max}$, then $^{\circ}D = [(T_{\max} - T_h)/2 \times (T_{\max} - T_h)/(T_{\max} - T_{\min})]$ and finally if $T_h \leq T_{\min}$, then $^{\circ}D = T_{\text{med}} - T_h$
4. The heat requirement (HR) was also calculated as a function of sum of the daily maximum temperatures, from the end of the chill period to the beginning of the pollen season (Galán et al. 2001):

$$HR = \sum T_{\max}$$

5. Finally we calculated the heat requirement by taking into account the days, after chilling, when the meteorological conditions enable plant physiological activity, either totally ($T_{\min} > T_h$) or partially ($T_{\max} > T_h > T_{\min}$) (referred to in this paper as vegetative activity). In this case, we considered the heat requirement as a function of the sum of the difference between the daily maximum temperature and the threshold temperatures:

$$HR = \sum (T_{\max} - T_h).$$

If $T_{\max}$ is less than or equal to the threshold temperature ($T_{\max} < T_h$), the values are set to 0.

Different threshold temperatures from 5.5 °C to 11 °C and from 5.5 °C to 8 °C were tested in increments of 0.5 °C, for chill and heat requirement respectively. The lowest standard deviation and coefficient of variation were used to identify the most adequate threshold temperature in the period of both the chilling and forcing temperatures, the best criterion for defining the onset of the pollen season and the most accurate method for determining the heat requirement.

Results

Phenological results

The dates of bud breaking, leaf unfolding and the start and end of flowering in deciduous *Quercus* species, and the start and end of flowering in perennial *Quercus* species, are shown in Table 1. *Q. robur* and *Q. faginea* had very similar dates for bud breaking and leaf unfolding. Both species, together with *Q. ilex*, started and ended flowering around the same time. *Q. robur* started to flower on 21 March, *Q. faginea* on 22 March and *Q. ilex* began flowering only 4 days later. *Q. suber* was the last to flower.

Q. robur and *Q. pyrenaica*, the most abundant *Quercus* species in the area, showed differences in their phenological behaviour. Thus, bud break in *Q. pyrenaica* occurred 23 days later and the flowering periods of the two species only overlap at the end of the *Q. robur* flowering period. The period between the onset of the differ-

Table 2 Chill requirements calculated according to the Aron (1983) method and the modified Aron method (selecting the days in which meteorological conditions did not enable any physiological activity – the vegetative activity method). *CH* chilling hours

Method	Years	Chill accumulation (<i>CH</i>) for a threshold of:								
		5.5 °C	6 °C	6.5 °C	7 °C	7.5 °C	8 °C	9 °C	10 °C	11 °C
Vegetative activity	1992–1993	836.1	841.2	846.6	849.1	855.7	862.5	877.9	919.8	1,013.9
	1993–1994	811.0	810.8	812.2	817.8	829.6	834.5	893.2	1,001.8	1,060.9
	1994–1995	830.8	835.2	844.5	846.4	850.6	855.2	877.2	868.4	982.3
	1995–1996		796.5	781.3	782	782.7	779.1	806.5	862.8	978.7
	1996–1997	812.7	830.0	835.0	839.6	859.0	878.4	979.9	1,085.6	1,143.9
	1997–1998							768.9	772.0	773.6
	1998–1999	816.8	819.2	797.9	818.9	853.3	877	978.6	1,138.9	1,140.8
	1999–2000		748.8	786.3	786.9	803.2	845.2	929.2	1,067.8	1,129.9
	Mean	821	812	815	820	833	847	889	965	1,028
	SD	11.29	31.63	27.47	27.31	29.84	34.06	75.04	128.61	123.67
	CV (%)	1.37	3.90	3.37	3.33	3.58	4.02	8.44	13.33	12.03
Aron (1983)	1992–1993	800.0	820.0	841.6	884.1	928.4	958.48	997.3	1,096.5	905.1
	1993–1994	705.1	733.4	770.6	825.8	868.9	934.5	1,104.7	1,001	469.7
	1994–1995	841.1	865.6	891.4	927.25	955.75	990.4	1,106.3	1,047.8	705.7
	1995–1996	775.1	788.5	802.9	818.2	834.48	851.5	908.45	940.2	1,032
	1996–1997	759.4	800.8	861.8	916.3	972.31	1,053.1	1,126	1,075.33	819.64
	1997–1998	656.3	653.8	663.5	730.8	769.2	806.49	905.4	1,044.1	1,093.2
	1998–1999	865.9	948.8	995.9	1,041.3	1,082.5	1,098.1	1,042.6	440.4	–1,073.7
	1999–2000	896.8	949	1,001.7	1,051.6	1,094.9	1,127.5	1,137.6	1,029.8	706.12
	Mean	787	820	854	899	938	978	1,041	959	582
	SD	81.12	101.27	113.02	110.22	114.06	113.55	94.68	215.02	697.89
	CV (%)	10.30	12.35	13.24	12.25	12.16	11.62	9.09	22.41	119.87

ent phenological phases was not homogeneous in the different species: there were 22 days between bud break and the start of flowering in *Q. robur*, 19 days in *Q. pyrenaica* and 15 in *Q. faginea*.

Chill requirement

Table 2 shows the chill accumulation (chilling units) obtained by using the two methods previously explained. The calculated chill requirements that take into account only the days in which meteorological conditions do not enable any physiological activity in the plant (vegetative activity method), show the smallest coefficient of variation. The results were satisfactory for threshold temperatures below 8 °C, and the small coefficient of variation was obtained for 5.5 °C. Nevertheless, we consider this temperature to be inadequate because in 3 of the 8 years (1995–1996, 1997–1998 and 1999–2000) temperatures below this threshold were not attained. The coefficient of variation for threshold temperatures between 6 °C and 8 °C was satisfactory. Only by using threshold temperatures above 9 °C was it possible to determine the chill accumulation by the vegetative activity method in 1997–1998. The smallest coefficient of variation when all the days below the threshold temperature were taken into account (Aron method) was obtained for 9 °C.

Taking 7 °C as the threshold temperature, the chilling period usually starts in mid-November (ranging from 13 November in 1999–2000 to 15 December in 1994–1995). The mean start date for accumulative chilling was 27 November. The chill requirement was reached during the first 15 days of January every year, except in

1993–1994, when the last day of accumulative chilling was 20 January, and in 1995–1996 when it was 16 December. On this date, in the latter case, the maximum and mean temperatures enabled either total or partial physiological activity in the plant. The mean end date of accumulative chilling was 7 January.

Heat requirements

The first day of the pollen season (calculated according to aerobiological data), or the date when a particular phenological event was observed, determined the end of the forcing period in each case.

When aerobiological data were used, the lowest standard deviation and coefficient of variation were obtained by taking the onset of the pollen season to be the day when the first pollen grains were registered on a continuous basis. Table 3 shows the results obtained for all threshold temperatures and criteria for determining the onset of the pollen season when the vegetative activity method was used. The same results were obtained by the Yang, Zalom, Ring and Galán methods, and the lowest standard deviation was always obtained with this criterion for the start of the pollen season.

Using this criterion to determine the onset of the pollen season, the heat requirement was calculated by means of the different criteria mentioned in Materials and methods and different threshold temperatures were assayed. The vegetative activity and Galán methods showed the lowest standard deviation and coefficient of variation (Table 4). Threshold temperatures displaying the lowest standard deviation and coefficient of variation

Table 3 Heat requirements (units defined in Materials and methods) according to different criteria for the start of the pollen season and threshold temperatures (5.5–8 °C). Criteria for the start of the pollen season: 1 the first day when pollen grains were regis-

tered on a continuous basis with no more than 2 days without pollen being detected; 95% (Torben 1991); 90% (Nilsson and Persson 1981). *SD* standard deviation, *CV* coefficient of variation

Threshold temperature (°C)	Criterion	Heat requirement										
		1992–1993	1993–1994	1994–1995	1995–1996	1996–1997	1997–1998	1998–1999	1999–2000	Mean	SD	CV (%)
5.5	1	624.5	458.3	597.3	611.7	594.8		658.3	657.1	600.3	67.7	11.28
	95%	767.5	600.5	813.8	1004.3	749.2		772.4	711.0	774.1	122.0	15.76
	90%	793.1	644.2	852.8	1093.0	770.5		815.7	729.3	814.1	140.0	17.20
6	1	593.0	433.8	564.8	574.7	567.3		626.8	628.6	569.9	65.6	11.51
	95%	731.0	571.0	773.8	951.3	717.7		736.4	681.0	737.5	114.3	15.50
	90%	755.6	613.2	811.8	1036.5	738.5		778.2	698.8	776.1	131.3	16.92
6.5	1	561.5	409.3	532.3	537.7	539.8		595.3	600.1	539.4	63.6	11.79
	95%	694.5	541.5	733.8	898.3	686.2		700.4	651.0	700.8	106.6	15.21
	90%	718.1	582.2	770.8	980.0	706.5		740.7	668.3	738.1	122.6	16.61
7	1	530.0	384.8	499.8	500.7	512.3		563.8	571.6	509.0	61.9	12.15
	95%	658.0	512.0	693.8	845.3	654.7		664.4	621.0	664.2	99.0	14.91
	90%	680.6	551.2	729.8	923.5	674.5		703.2	637.8	700.1	113.9	16.27
7.5	1	499.7	360.5	467.3	463.7	484.8		532.3	543.1	478.8	60.3	12.60
	95%	622.7	482.7	653.8	792.3	623.2		628.4	591.0	627.7	91.5	14.57
	90%	644.3	520.4	688.8	867.0	642.5		665.7	607.3	662.3	105.3	15.89
8	1	469.2	337.0	434.8	426.9	457.3		500.8	514.6	448.7	58.7	13.09
	95%	587.2	454.2	613.8	739.5	591.7		592.4	561.0	591.4	83.9	14.18
	90%	607.8	490.4	613.8	810.7	610.5		628.2	576.8	619.7	96.1	15.50

Table 4 Heat requirements calculated by different methods and using different threshold temperatures to identify the process that showed the lowest standard deviation. The start of the pollen

season was considered the first day when pollen grains were registered on a continuous basis

Years	Heat requirements										
	Vegetative activity			Yang		Zalom			Ring		Galán 7 °C
	6 °C	6.5 °C	7 °C	6 °C	6.5 °C	6 °C	7 °C	7.5 °C	6 °C	6.5 °C	
1992–1993	593.0	561.5	530.0	156.9	125.4	288.6	179.5	163.2	168.4	142.1	971.0
1993–1994	433.8	409.3	384.8	161.6	138.1	189.4	150.5	137.2	158.2	137.9	727.8
1994–1995	564.8	532.3	499.8	223.4	190.9	257.8	209.3	177.5	231.2	202.8	954.8
1995–1996	574.7	537.7	500.7	268.8	227.8	301.4	250.2	215.9	281.4	248.8	1,018.7
1996–1997	567.3	539.8	512.3	252.1	224.6	268.1	223.8	202.9	253.3	226.8	897.3
1997–1998											
1998–1999	626.8	595.3	563.8	218.7	187.2	270.2	216.3	192.0	222.4	194.7	1,004.8
1999–2000	628.6	600.1	571.6	225.7	197.2	268.1	226.5	207.0	239.8	217.8	970.6
Mean	569.9	539.4	509.0	215.3	184.5	263.4	208.0	185.1	222.1	195.8	935.0
SD	65.6	63.6	61.9	42.2	39.5	35.8	33.1	27.8	44.4	41.9	99.3
CV (%)	11.5	11.8	12.2	19.6	21.4	13.6	15.9	15.0	20.0	21.4	10.6

differed with the method used, but were always between 6 °C and 7.5 °C. The lowest standard deviation and coefficient of variation were obtained with 6 °C and 6.5 °C when the vegetative activity method was used, and with 7 °C by the Galán method.

According to the phenological observations, Table 5 shows the heat requirements of the different species and phenological events studied and calculated by the two methods. Finally the heat requirement calculated by using aerobiological data from 2001 are displayed in Table 6. In this case, the different criteria for defining the onset of the pollen season were evaluated and the results

compared with those obtained using phenological data (Table 5). Similar values were obtained for the flowering of *Q. robur*, *Q. ilex* and *Q. faginea*, and the onset of the pollen season defined as the date when 2.5% of the annual pollen total is reached in both the Galán and vegetative activity methods.

Aerobiological results

Since the pollen morphology of the different *Quercus* species is very similar, it is not easy to distinguish the

Fig. 2 Concentrations of pollen grains of the different *Quercus* species during the period of the phenological study. Line under graph start of the pollen season estimated using different criteria. Lines above graph phenological phases of the different *Quercus* species (1 bud break, 2 leaf unfolding, 3 flowering)

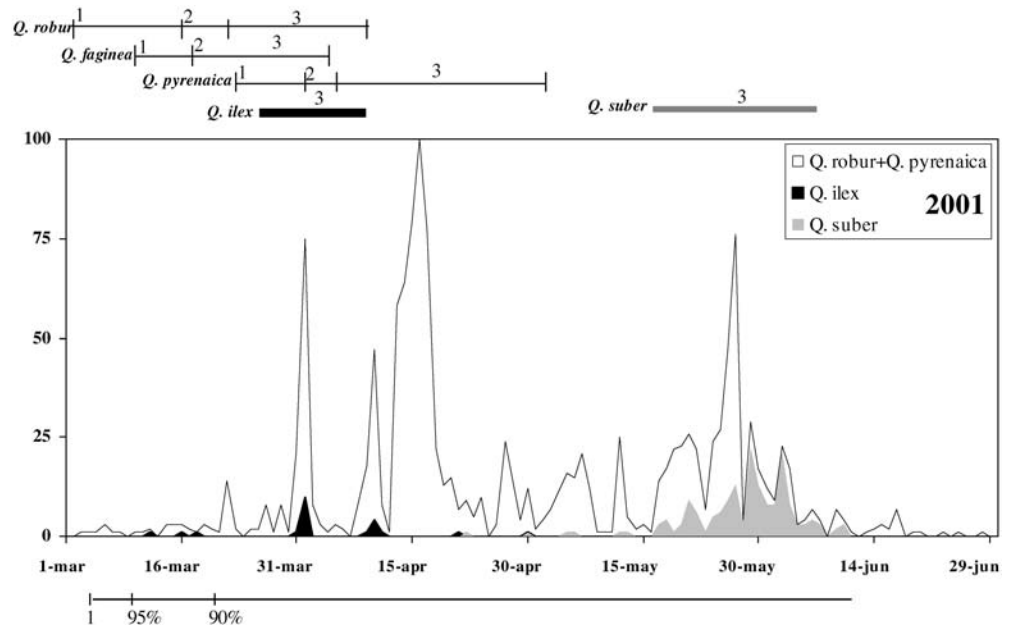


Table 5 Heat requirements of the different species and phenological events studied and calculated by means of the vegetative activity (VA) and Galán methods

Species	Heat requirements for:								
	Bud break			Leaf unfolding			Onset of flowering		
	Date	VA	Galán	Date	VA	Galán	Date	VA	Galán
<i>Quercus robur</i>	27/2/01	401	758	15/3/01	555	1,024	21/3/01	618	1,129
<i>Quercus faginea</i>	7/3/01	468	881	14/3/01	540	1,002	22/3/01	632	1,150
<i>Quercus pyrenaica</i>	22/3/01	632	1,150	5/4/01	784	1,401	10/4/01	851	1,502
<i>Quercus ilex</i>							25/3/01	659	1,198
<i>Quercus suber</i>							15/5/01	1295	2,191

Table 6 Dates of the start of the aerobiological pollen season calculated with different criteria (defined as in Table 3), and heat requirements calculated by the Galán and vegetative activity (VA) methods

Criterion	Date	VA	Galán
1	3/3/01	477	897
95%	20/3/01	607	1,111
90%	29/3/01	696	1,263

importance of each species in the pollen curve. Nevertheless, taking into account the size and sculptural elements of the exine, we identified the pollen of *Q. ilex* and *Q. suber* (Fig. 2). At the beginning of the pollen season, *Q. ilex* pollen was captured in limited quantities. *Q. suber* pollen was present continuously from mid-May and represented the majority of the pollen captured at the end of the pollen season.

The largest quantity of *Quercus* pollen was registered when *Q. robur* and *Q. pyrenaica* were flowering, and the highest value was obtained during the flowering period of *Q. pyrenaica*. However, it is necessary to point out

that pollen of *Q. robur* and *Q. pyrenaica* was captured after all the trees of both species near the spore-trap had finished their flowering period.

Discussion

The first species to flower was *Q. robur*, which almost coincided with the flowering of *Q. ilex* and *Q. faginea* (Table 1 and Fig. 2). The end of flowering in the three species took place around the same time. When the flowering of *Q. robur* was ending, *Q. pyrenaica* started flowering and its flowering period was the longest. When the rest of the species finished their flowering period, *Q. suber* flowered. This was the last species to flower and its period did not overlap with any other *Quercus* species (Fig. 2). Despite the different dates on which *Q. robur* and *Q. pyrenaica* flowered (Table 5), in both species the heat requirement calculated between bud break and leaf unfolding and between leaf unfolding and the start of flowering were very similar (154 and 63 heat units between bud break and leaf unfolding in *Q. robur* calculated by the vegetative activity method and 152 and

Table 7 Mean of the maximum, medium and minimum temperatures of the period from 15 November to 7 January

Years	Temperature 15 November–7 January (°C)		
	Maximum	Mean	Minimum
1992–1993	13.1	9.2	5.3
1993–1994	13.1	9.1	5.0
1994–1995	14.1	9.4	5.0
1995–1996	14.8	10.8	6.8
1996–1997	12.5	8.5	4.5
1997–1998	14.5	10.8	7.2
1998–1999	12.9	8.6	4.3
1999–2000	12.5	7.9	3.3
2000–2001	14.5	11.2	7.8

67 units in *Q. pyrenaica*). However, *Q. pyrenaica* has a higher heat requirement to produce bud break.

Probably the clear Eurosiberian character of *Q. robur* determines a lower heat requirement to go back to a phase of active growth in the spring. The heat required by *Q. faginea* between bud break and leaf unfolding was lower and, by contrast, was higher between leaf unfolding and the start of flowering (72 and 92 units respectively). The heat requirements for inducing bud break and leaf unfolding in *Q. robur* and *Q. faginea* in 2001 were very similar; those triggering flowering were very similar in *Q. robur*, *Q. faginea* and *Q. ilex*, with *Q. robur* showing the lowest value (Table 5). The heat requirements for inducing any phenological event in *Q. pyrenaica* were higher. *Q. suber* had highest heat requirement to induce flowering.

Since *Q. ilex* was less in evidence in the area surrounding the spore-trap, and also because strong and abundant precipitation was registered during the flowering period, only a limited number of *Q. ilex* pollen grains were captured. The morphology of *Q. faginea* pollen prevented us from separating it out in the counting, nevertheless its presence is rare in our territory and therefore its importance in the pollen curve can be considered insignificant. Pollen grains of *Q. suber* rise at the end of May, though with low concentrations. *Q. robur* and *Q. pyrenaica* are the most widespread species in the area and the most important sources of *Quercus* pollen in the air. Pollen grains of *Quercus* remained in the air even when no *Quercus* trees close to the spore-trap were flowering, a period ranging from the end of flowering of *Q. pyrenaica* to the start of flowering of *Q. suber* (Fig. 2). However, during that time flowering oak trees located higher up (400–500 m above sea level) but far away from the spore-trap were observed. The abundance of both species of *Quercus* in the Ourense surroundings, located at different altitude and directions, is confirmed by the spore trap records capturing *Quercus* pollen transported from a much wider area. The low-lying valley surrounded by mountains makes this transportation along the river valleys easier. Therefore, although the characteristics of the pollen grains hinder transportation, this does seem to occur in our area.

The lowest coefficients of variation and standard deviation obtained using the vegetative activity method confirm that it is a good method for calculating chilling requirement in Ourense. Temperatures below 8 °C are considered adequate for accumulating chilling. However, in warm winters higher temperatures must be used. Therefore, in 1997–1998 and 2000–2001 the temperatures registered during the chilling period were higher, specially the minimum temperatures (Table 7), and chilling below 8 °C was not possible. The average chilling required, using 7 °C as the threshold temperature, when aerobiological data was employed, varied between 820 and 899 hours depending on the method (vegetative activity and Aron methods respectively) (Table 2).

The onset of the pollen season, considered as the first day when pollen grains were registered on a continuous basis with no more than 2 days without pollen being detected, showed the lowest coefficient of variation and standard deviation (Table 3). The start of the pollen season, based on the date when 1 pollen grain/m³ is recorded and when the subsequent days also have 1 or more pollen grains/m³, was pointed out by Garcia-Mozo et al. (2000) as a useful criterion for making predictions.

The heat requirement calculated by the vegetative activity method (taking into account when the meteorological conditions enable plant physiological activity, either totally or partially) or by the Galán method showed the lowest coefficient of variation and standard deviation (Table 4). The average heat requirement calculated with the vegetative activity method was 509 units when 7 °C was used as the threshold temperature, and 935 units when the sum of accumulated maximum temperatures (above 7 °C) was used in the Galán method.

Chill and heat requirements for *Quercus* were previously studied by Garcia-Mozo et al. (2000) and Rodríguez-Rajo et al. (2000) in Spain. The chilling and heat required by trees vary according to species and climatic conditions. Threshold temperatures should also be adjusted for specific environmental conditions of the area and species; therefore, the differences in such factors enable us to explain the differences observed between the results obtained in Cordoba (Garcia-Mozo et al. 2000) and those in our area. *Q. ilex* subsp. *ballota* is the first *Quercus* species to flower in the south of Spain, while in Ourense *Q. robur* is the first. Rodríguez-Rajo et al. (2000) used different threshold temperatures and methods, which accounts for the differences observed between the two studies. The results obtained by Garcia-Mozo et al. (2002) for predicting the onset of the *Quercus* pollen season in different climate zones in Spain indicate that a different threshold temperature provides the most accurate model for each site. Even the same species in different localities show adaptations to different environmental conditions, as pointed out for *Q. humilis* in France (Chuine and Cour 1999) and for *Q. ilex* subsp. *ballota* in Spain (Garcia-Mozo et al. 2002).

Similar values for the heat requirement were obtained for the flowering of *Q. robur*, *Q. ilex* and *Q. faginea*, and

the onset of the pollen season, defined as being when 2.5% of the pollen total was reached (Tables 5, 6). This seems to contradict the result obtained using aerobiological data. Nevertheless, we made observations that provided a better understanding of the data: some other oak trees were observed flowering before the trees studied were in flower, which means that aerobiological data are a good tool for studying the flowering phenophase and allow to detection of the first trees in flower. Nevertheless, new studies with a higher number of trees will be necessary to make the results more representative of the total populations in the area.

On the other hand, the influence of biotic factors on pollen production and on the onset of the pollen season was verified. *Quercus* started flowering at the beginning of March, and in 2 or 3 days we observed a high number of galls growing on the catkins. Water is very important to the development of galls, so in a few days the catkins dried and pollen production ended. This event especially affected trees that had flowered before. The insect responsible for this event was *Neuropterus*, which produces lenticular galls in the autumn. A generation of asexual *Neuropterus* emerges from these galls and the following spring they lay eggs on the buds, resulting in the growth of spherical galls (like raspberries) in both young leaves and catkins. In a short time new males and females (sexual generation) emerge and produce lenticular galls (Dajoz 1999). The number of galls changes every year and temperature and rainfall are the most important factors in such changes. Limited precipitation usually produces a low number of galls (De Frutos 1986). The removal of pollen grains from the atmosphere is frequently indicated in aerobiological studies. This occurs of course, but now we can also consider the possibility of other factors having an important effect on pollen monitoring and on the start of the pollen season when such events occur at the beginning of flowering. Therefore, the first continuous registering of pollen grains is a good indication of the flowering event that is taking place. Different factors, such as rainfall and biotic factors, can delay the onset of the pollen season calculated by means of aerobiological data, especially when a certain percentage of the annual total is eliminated.

Conclusions

The vegetative activity method is a good way of calculating chilling requirements in Ourense. The vegetative activity and Galán methods were the most accurate for calculating the heat requirement. Temperatures above 8 °C and between 6 °C and 8 °C are good threshold temperatures for calculating chill and heat requirements respectively.

Q. robur showed the lowest heat requirement for triggering flowering and this value was similar to those for *Q. faginea* and *Q. ilex*. The heating needed to induce any phenological event in *Q. pyrenaica* was higher and *Q. suber* had the highest heat requirement for inducing flowering.

Q. robur is the first *Quercus* species to flower. Therefore, the use of aerobiological data in our territory is a good tool for studying the phenology of this *Quercus* species.

The onset of the pollen season, considered as the first day when pollen grains are registered on a continuous basis with no more than 2 days without pollen being detected, is a useful criterion for determining the flowering phenophase from aerobiological data.

The *Quercus* pollen curve is according to the phenology of the *Quercus* trees in the area and reflects the influence of the medium-distance transportation of *Q. robur* and *Q. pyrenaica* pollen, from areas 10–30 km away from the spore trap, on the concentrations of *Quercus* pollen registered in Ourense. This can explain the quantities of *Quercus* pollen registered when no *Quercus* trees surrounding the city are in flower.

Climatic and biotic factors should be taken into account for a correct interpretation of the aerobiological results. Both factors can delay the onset of the pollen season when a percentage of the pollen is removed at the beginning of the season as a criterion for defining it.

Phenology is a useful tool in aerobiological studies and aids our understanding of the aerobiological results when we consider the different factors that are involved therein. More extensive studies should be conducted in order to improve interpretation.

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