

Alternative statistical methods for interpreting airborne Alder (*Alnus glutinosa* (L.) Gaertner) pollen concentrations

Zulima González Parrado · Rosa M. Valencia Barrera ·
Carmen R. Fuertes Rodríguez · Ana M. Vega Maray ·
Rafael Pérez Romero · Roberto Fraile ·
Delia Fernández González

Received: 28 July 2008 / Accepted: 3 September 2008 / Published online: 14 October 2008
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Abstract This paper reports on the behaviour of *Alnus glutinosa* (alder) pollen grains in the atmosphere of Ponferrada (León, NW Spain) from 1995 to 2006. The study, which sought to determine the effects of various weather-related parameters on *Alnus* pollen counts, was performed using a volumetric method. The main pollination period for this taxon is January–February. Alder pollen is one of the eight major airborne pollen allergens found in the study area. An analysis was made of the correlation between pollen counts and major weather-related parameters over each period. In general, the strongest positive correlation was with temperature, particularly maximum temperature. During each period, peak pollen counts occurred when the maximum temperature fell within the range 9°C–14°C. Finally, multivariate analysis showed that the parameter exerting the greatest influence was temperature, a finding confirmed by Spearman correlation tests. Principal components analysis suggested that periods with high pollen counts were characterised by high maximum temperature, low rainfall

and an absolute humidity of around 6 g m⁻³. Use of this type of analysis in conjunction with other methods is essential for obtaining an accurate record of pollen-count variations over a given period.

Keywords *Alnus* pollen · Aerobiology · Meteorological parameters · Correlations · Principal components analysis

Introduction

Alnus glutinosa (L.) Gaertner (alder) is one of the species, along with *Betula alba* L., *Salix atrocinerea* Brot and *Frangula alnus* Miller (Rivas-Martínez 1987), forming part of the riparian forest around Ponferrada, Spain. Its geographical range extends from the Eurosiberian region to the Mediterranean (Rodríguez-Rajo et al. 2004).

Alder was selected for this study because it is widespread in Ponferrada, but also because its pollen is one of the eight major airborne pollen allergens in north-western Spain (Valencia-Barrera et al. 1999; Vega-Maray et al. 2002); *Alnus* sp. is a major allergen both in its own right and because of cross-reactivity with pollen grains of other genera belonging to the Betulaceae and Fagaceae families (Mathiesen et al. 1991; Subiza et al. 1998).

Over recent years, numerous studies have demonstrated the existence of considerable regional differences in both the timing and the duration of pollen seasons. These differences are clearly associated with climate and weather conditions, as well as with local geography, geomorphology and height above sea level (Frenguelli et al. 1991; Emberlin et al. 1994, 2000; Corden et al. 2000; Zanoti and Puppi 2000; Jato et al. 2002a; Kasprzyk 2008). Correlations have also been reported between various weather-related param-

Z. González Parrado (✉) · R. M. Valencia Barrera ·
C. R. Fuertes Rodríguez · A. M. Vega Maray · R. Pérez Romero ·
D. Fernández González
Department of Biodiversity and Environmental Management
(Botany), University of León,
Campus de Vegazana s/n,
24071 León, Spain
e-mail: zgonp@unileon.es

R. Fraile
Department of Applied Physics, University of León,
Campus de Vegazana s/n,
24071 León, Spain

eters and airborne *Alnus* pollen counts (Aira et al. 1998; Iglesias et al. 2003 and Rodríguez-Rajo et al. 2003a).

Alnus is a winter-flowering tree, and previous studies have highlighted a significant correlation between the pollen-season start date and temperature during the period preceding the main pollen season (Frenguelli et al. 1993; Jato et al. 2000; Rodríguez-Rajo et al. 2004, 2006, González Parrado et al. 2006; Emberlin et al. 2007).

The aim of this study was to analyse airborne *A. glutinosa* pollen counts in Ponferrada (NW Spain) in order to determinate seasonal variations and identify the main weather parameters influencing airborne pollen counts. Multivariate analysis was used to chart variations in pollen counts over given periods. Use of this type of analysis in conjunction with other methods is essential for obtaining an accurate record of pollen-count variations over a given period.

Materials and methods

Study area

The city of Ponferrada is located 541 m above sea level in the northwestern Iberian Peninsula (42° 33'N; 6° 35'W). In biogeographical terms, Ponferrada is located in the Mediterranean region, upper mesomediterranean bioclimatic stage, with a lower subhumid ombroclimate (del Río-González 2005).

Aerobiological survey

Airborne pollen was collected using a 7-day Lanzoni pollen trap (Hirst 1952). Pollen counts were monitored from 1995 to 2006, using the standard sampling procedure proposed by Domínguez (1992). Pollen data are given as pollen grains per cubic meter of air.

The beginning and end of the main pollination period (MPP) were determined using Andersen's method (Andersen 1991), which defines the pollen season as the period in which 95% of the total catch occurs.

Pollen counts were measured from October (the previous year) to September (in the year of pollination) since the alder usually flowers in late December or early January.

Meteorological data

The following meteorological parameters were analysed: maximum (MT), minimum (mT), mean (MeT) and wet-bulb (WT) temperature, dew point (DP), precipitation (P), relative humidity (RH), absolute humidity (AH), wind speed (WS), wind direction [N-NE (Q1); S-SE (Q2);

S-SW (Q3) and N-NW (Q4)], frequency of calm (C) and evaporation (EV). These data were supplied by the Ponferrada weather station (Spanish Meteorology Network).

Statistical analysis

Two types of statistical analysis were performed:

- I. Spearman's test, using the SPSS 15.0 statistical package, in order to establish potential correlations between meteorological factors and daily pollen counts; analysis was performed over two different pollen periods:
 - a) MPP: from the beginning to the end of the pollen season.
 - b) Pre-peak: from the start of the MPP to the day of peak concentration.
- II. Principal components analysis (PCA), using XLSTAT v. 7.0; PCA was performed on the correlation matrix obtained from the standardised data for weather-related parameters and pollen counts during the MPP. The first principal component accounts for the largest portion of the variability of the original data set, the second principal component accounts for the second largest, and so on (Chao et al. 2002). Only parameters with levels > 0.45 were considered.

Total pollen counts and mean values for meteorological parameters during the MPP were used, except for rainfall, for which the total value was used.

Results

Aerobiological survey

Over the study period as a whole, a total of 14,815 (1996–2006) *Alnus* pollen grains were counted—this figure represents only 5% of the total pollen count for the period. The mean annual *Alnus* pollen count was 1,374 pollen grains m⁻³. The highest pollen counts were recorded in 1998–1999 (2,541 grains m⁻³), and the lowest counts in 1997–1998 (304 grains m⁻³) (Table 1).

The MPP for *Alnus* generally ran from January to the second half of February (Table 1). The mean duration of the MPP was 49 days; MPP duration ranged from 30 days (2001–2002) to 67 days (1995–1996). The peak pollen season for *Alnus* tended to occur in the 5th or 6th week of pollination, corresponding to the first half of February (Table 1).

There was some year-on-year variation in the pollen-season start date; the positive slope of the line in Fig. 1 indicates a gradual and light delay in pollen-season onset.

Table 1 Chief characteristics of the main pollination period (MPP) for *Alnus glutinosa* from 1995 to 2002 in the city of Ponferrada

	Start date of MPP	End date of MPP	Duration of MPP (days)	Grains of pollen (air m ⁻³)	Peak day
1995–1996	31 December 1995	6 March 1996	67	662	27 January 1996
1996–1997	25 December 1996	21 February 1997	59	811	1 February 1997
1997–1998	4 January 1998	23 February 1998	51	304	18 January 1998
1998–1999	22 January 1999	27 February 1999	37	2,541	7 February 1999
1999–2000	16 January 2000	22 February 2000	38	2,155	6 February 2000
2000–2001	19 December 2000	12 February 2001	56	1,340	4 January 2001
2001–2002	25 January 2002	23 February 2002	30	1,942	30 January 2002
2002–2003	23 December 2002	20 February 2003	60	1,552	29 January 2002
2003–2004	31 December 2003	17 February 2003	49	1,499	12 January 2004
2004–2005	22 January 2004	10 March 2004	48	521	10/11 February 2005
2005–2006	25 January 2006	04 March 2006	39	838	11 February 2006

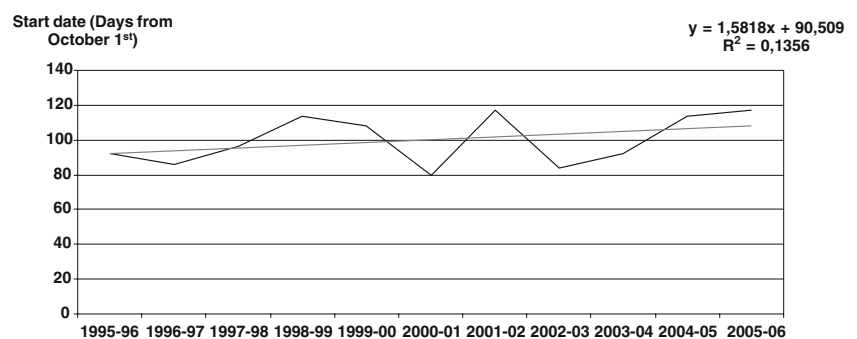
Statistical analysis

Spearman's test

A greater degree of correlation was obtained with the pre-peak period than with the MPP. In general, the strongest positive correlation was with temperature, mainly maximum temperature. Maximum daily temperatures of between 9°C and 14°C during the MPP were associated with peak pollen count (Fig. 2). When all study years were taken into account, this correlation decreased; the strongest correlation with absolute humidity was recorded in 2001–2002 (−0.94).

Rainfall has a considerable influence in cleaning air of particles, including pollen grains; however, there was no significant correlation between rainfall and pollen counts for most years, with the exception that rainfall in 1995–1996 displayed a significant negative correlation with pollen counts. Only in 2003–2004 did we obtain a positive significant correlation with rainfall; during this period, rainfall occurred largely at night, when pollen levels were low, and therefore atmospheric cleansing was less important (Table 2). Periods characterised by high pollen counts, such as 1999–2000, were associated with low rainfall (13 mm). Conversely, pollen counts in 2000–2001 did not exceed 1,340 pollen grains, whereas rainfall was around 348 mm (Table 3).

Fig. 1 Start dates (black line) and regression line (grey line) for the *Alnus glutinosa* pollen-season in the city of Ponferrada, NW Spain



Correlations with relative or absolute humidity or with evaporation were not significant for most study years. Correlations with wind-related parameters were not significant, although peak pollen counts tended to occur when the wind was blowing from south-south-west, and the strongest correlations were recorded with this wind direction (Table 2).

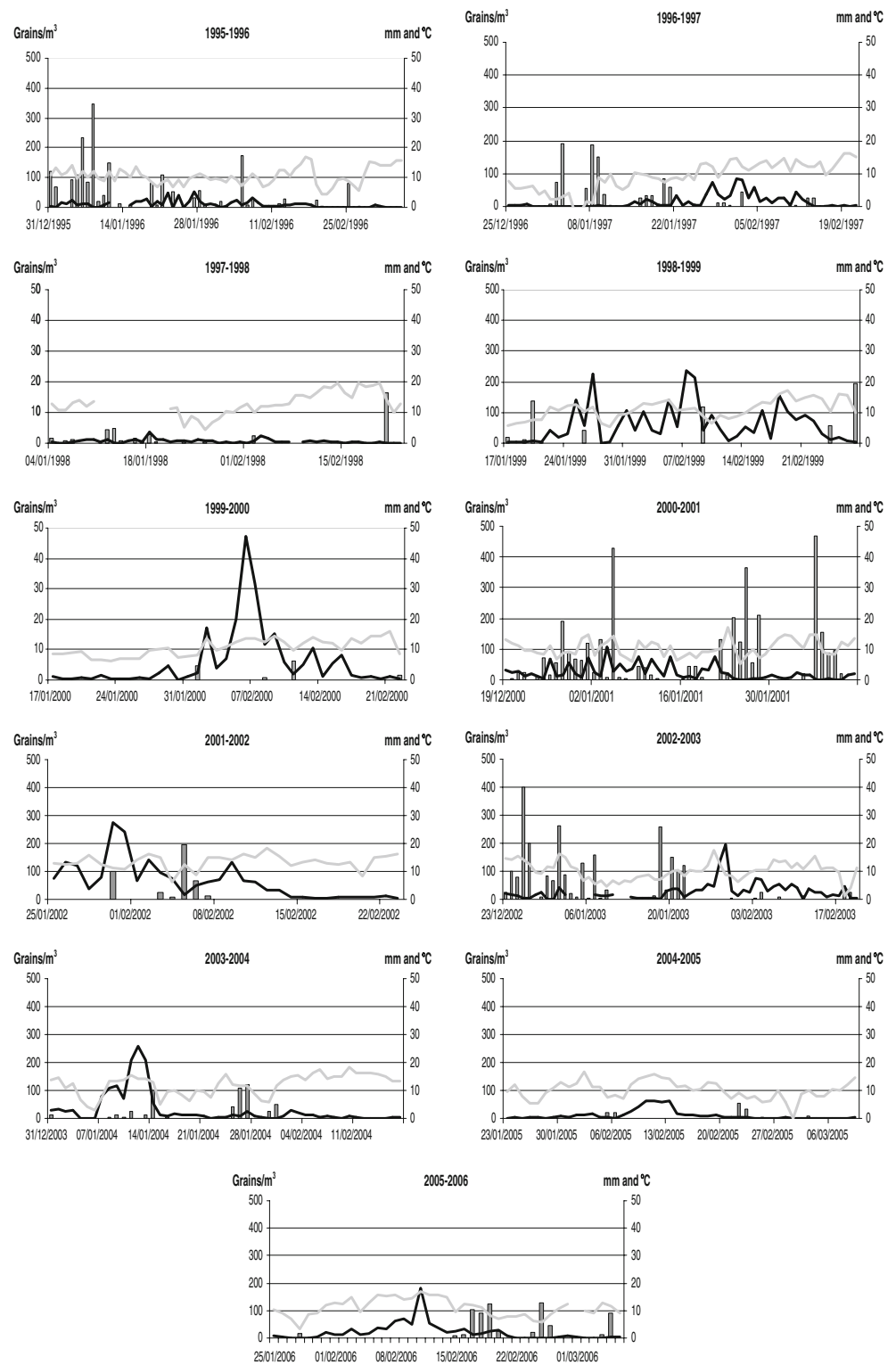
Principal components analysis

Table 4 shows that the first three PCA factors (F1, F2 and F3) accounted for over 78% of total variance, the three axes being correlated as follows:

- Factors 1 (F1): All temperature data (except maximum temperature), rainfall, relative and absolute humidity, wind from S-SW and evaporation.
- Factors 2 (F2): Wind speed, wind from N-NE and N-NW, calms and evaporation.
- Factors 3 (F3): Pollen concentration, maximum temperature, rainfall, absolute humidity and wind from N-NE.

The results of PCA can be plotted on two-dimensional graphs. Figure 3a shows factors 1 and 2: most study periods appear on the positive side of the F1 axis. These periods

Fig. 2 Variations in maximum temperature (*grey lines*, °C), rain (*bars*, mm) and daily average of *Alnus* pollen concentrations (*black lines*, grains m^{-3})



were characterised by high temperatures, wind blowing from the S-SW and lower evaporation. In contrast, the periods with the lowest mean temperatures [2004–2005 (3.7°C) and 2005–2006 (4.7°C)] appear on the negative side of the F1 axis.

The negative side of the F2 axis includes periods with low values for calms, while the positive side of this axis includes periods with high values. Figure 3b shows factors 1 and 3: distribution of pollen counts over pollination periods is more readily appreciable.

Table 2 Significant Spearman's correlation coefficients between *Alnus* pollen counts and weather-related parameters for MPP and pre-peak (PP). *MT* Maximum temperature; *mT* minimum temperature; *MeT* mean temperature; *WT* wet-bulb temperature; *DP* dew point; *P* precipitation; *RH* relative humidity; *AH* absolute humidity; *WS* wind speed; *Q1*, *Q2*, *Q3*, *Q4* wind direction N-NE, S-SE, S-SW, and N-NW, respectively; *C* frequency of calm, *EV* evaporation

	1995-1996		1996-1997		1997-1998		1998-1999		1999-2000		2000-2001		2001-2002		2002-2003		2003-2004		2004-2005		2005-2006		1995-2006	
	MPP	PP	MPP	PP	MPP	PP	MPP	PP	MPP	PP	MPP	PP	MPP	PP	MPP	PP	MPP	PP	MPP	PP	MPP	PP	MPP	PP
MT	-	-	0.58**	0.79**	-	-	0.50**	0.67**	-	0.72**	0.61**	-	-	-0.85*	0.27*	-	-	0.73**	0.63**	0.78**	0.74**	0.93**	-	0.64**
mT	0.27*	-	0.38**	0.48**	-	-	-	-	0.63**	0.62**	-	-	0.54**	-	-	-	0.45**	0.75**	-	-	-	-	0.12**	0.20**
MeT	-	-	0.52**	0.68**	-	-	-	0.46*	0.68**	0.75**	-	-	-	0.82**	-	-	0.41**	0.80**	0.66**	0.80**	0.66**	0.87**	0.25**	0.49**
WT	0.29*	-	0.46**	0.57**	-	-	-	-	0.65**	0.73**	-	-	0.45*	-	-	-	0.47**	0.77**	0.50**	0.67**	0.33*	0.55**	-	0.35**
DP	0.26*	-0.49*	0.39**	0.48**	-	-	-	-	0.60**	0.62**	-	-	0.54**	-	-	-	0.46**	0.69**	0.46**	-	-	-	-0.14**	0.23**
P	-	-	-	-	-	-	-0.36*	-	-	-	-	-	-	-	-	-	0.39**	0.69**	-0.29*	-	-	-	-	-0.14*
RH	-	-	0.28*	-0.44**	-	-	-	-	-	-	-	-0.60*	0.49**	-	-	-	-	-	-	-	-	-	-	-0.26**
AH	0.25*	-	0.39**	0.47**	-	-	-	-	0.58**	0.60**	-	-	-	-0.94**	-	-	0.45**	0.69**	0.42**	-	-	-	0.25**	0.24**
WS	-	-	-	-	-	-	-	-	0.35*	-	-	-	-	-	0.30*	-	0.60**	0.73**	-	-	-0.45**	-0.70**	0.14**	0.19**
Q1	-	-	-	-	-	-	-0.43**	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.46**	-	-0.10*	-
Q2	-	-	-	-	-	-	-0.43**	-	-	-	-	-	-	-	-	-	-	-0.69**	-	-	-	-	-0.65*+	-
Q3	-	-	-	0.33*	-	-	0.43**	0.49*	0.61**	0.58**	-	-	-	-	-	-	0.49**	0.58*	-	-	-	-	0.20**	0.27**
Q4	-0.44*	-	-	-	-	-	-	0.43*	-	-	-	-	-	-	-	-	-0.58**	-	-0.31*	-0.49*	-	-	0.23**	-
C	-	-	-	-	-	-	-	-	-0.44**	-0.47*	-	-	-	-	-	-	-	-0.62*	-	-	0.39*	0.56*	0.12**	-0.17**
EV	-	-	-	0.46**	-	-	0.42**	0.48*	-	-	-	-	-0.43**	-	-	-	-	0.73**	-	-	-	-	0.12**	0.31**

* $P < 0.05$, ** $P < 0.001$

Table 3 Mean *Alnus* pollen counts and mean values for meteorological parameters, except rainfall (expressed as the total over each study period)

	Total pollen	MT	mT	MeT	WT	DP	P	RH	AH	WS	Q1	Q2	Q3	Q4	C	EV
1995–1996	662	10.5	2.9	6.7	4.9	3.1	205	76.7	5.9	4.9	3.6	4.5	5.9	2.7	7.3	1.6
1996–1997	811	9.1	1.6	5.4	4.2	3.0	109	83.1	5.9	4.0	1.9	7.4	4.9	2.9	6.8	1.0
1997–1998	305	13.0	2.8	7.9	6.2	4.0	41	77.2	6.4	5.3	3.7	6.6	4.9	2.3	6.5	1.5
1998–1999	2,563	11.1	1.1	6.1	4.3	2.1	58	78.0	5.7	4.4	1.4	6.8	6.6	3.7	5.6	1.3
1999–2000	2,142	10.7	1.5	6.1	4.5	2.8	13	81.8	6.0	3.8	1.5	6.7	5.8	4.0	6.1	1.4
2000–2001	1,340	10.3	4.5	7.4	6.0	4.5	348	84.2	6.6	8.3	5.7	5.1	7.5	2.3	3.3	1.3
2001–2002	1,942	13.5	1.6	7.6	5.8	3.6	41	76.7	8.2	6.3	3.2	6.7	4.9	7.9	1.2	2.1
2002–2003	1,333	10.2	1.8	6.0	4.3	2.2	80	79.4	5.9	6.6	2.6	4.6	5.9	8.7	2.2	1.8
2003–2004	1,499	12.1	2.2	7.2	5.5	3.7	52	81.0	6.4	3.5	1.6	0.5	7.3	2.0	12.6	1.6
2004–2005	518	10.1	-2.7	3.7	1.2	-2.5	14	64.3	4.1	4.9	4.6	5.4	4.5	6.1	3.3	2.7
2005–2006	845	10.8	-1.5	4.7	2.0	-0.9	2	69.5	4.6	5.0	1.3	1.7	4.6	5.6	10.9	2.0

The central part of the plot contains a group of periods characterised by high pollen counts and maximum temperature, low rainfall and absolute humidity of around 6 g m^{-3} . This was the case during 1998–1999 (2,563 pollen grains), 1999–2000 (2,142 pollen grains), 2002–2003 (1,625 pollen grains), 2003–2004 (1,575 pollen grains), but not during 1997–1998, which—although also located in the central part—had very low pollen counts (328 pollen grains). This may be because mean temperatures were high.

The other part of the plot comprises 1995–1996 and 1996–1997. This part lies beneath the previous group because of heavy rainfall during this period; conditions were conducive to high pollen counts, but these were cancelled out by heavy rainfall.

At the bottom on the negative side of the F3 axis is 2000–2001, the period of greatest rainfall (348 mm). At the

top of the plot is 2001–2002, characterised by high pollen counts and high absolute humidity (8.2 g m^{-3}).

Finally, 2004–2005 and 2005–2006 are shown on the negative side of the F1 and F3 axis because these periods had low temperatures and very low absolute humidity (4.6 and 4.1 g m^{-3} , respectively).

Discussion

Aerobiological survey

Alnus pollen is a major component of airborne particle content in Ponferrada, and accounts for between 2.3% and 7.4% of total pollen levels. Similar percentages have been reported for other sites in north-western Spain, e.g. Santiago

Table 4 Results of principal components analysis (PCA): factors from F1 to F10

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	8.84	3.00	2.30	1.65	1.21	0.59	0.18	0.14	0.06	0.02
Variability (%)	49.11	16.64	12.80	9.15	6.74	3.26	1.02	0.80	0.34	0.12
Storage %	49.11	65.75	78.55	87.70	94.44	97.71	98.73	99.53	99.88	100.00
Pollen	0.29	0.15	0.47	0.53	0.54	-0.29	0.09	0.07	0.01	0.06
MT	0.32	-0.10	0.74	-0.54	-0.06	-0.19	0.12	-0.02	0.01	-0.03
mT	0.98	-0.02	-0.15	-0.02	-0.04	0.05	-0.03	-0.05	-0.09	0.04
MeT	0.91	-0.07	0.26	-0.29	-0.07	-0.06	0.04	-0.05	-0.06	0.01
WT	0.97	-0.04	0.19	-0.13	-0.10	-0.03	-0.04	-0.03	-0.01	-0.02
DP	0.98	0.07	0.10	0.00	-0.12	0.06	-0.06	0.03	-0.01	-0.01
P	0.60	-0.27	-0.70	-0.00	0.14	-0.04	0.04	0.21	-0.10	-0.00
RH	0.88	0.24	-0.08	0.34	0.02	0.16	-0.09	-0.01	0.15	0.01
AH	0.80	-0.23	0.48	-0.05	-0.01	0.07	-0.10	0.24	0.05	-0.04
WS	0.34	-0.79	-0.23	-0.11	0.29	0.17	0.27	-0.03	0.07	-0.01
Q1	0.18	-0.76	-0.47	-0.29	-0.10	-0.25	-0.10	-0.01	0.08	0.02
Q2	0.17	-0.42	0.15	0.64	-0.55	-0.23	0.06	-0.02	-0.02	-0.00
Q3	0.66	0.25	-0.29	0.00	0.57	-0.26	-0.10	-0.12	-0.00	-0.06
Q4	-0.43	-0.55	0.45	0.19	0.33	0.39	-0.08	-0.04	-0.06	-0.01
C	-0.09	0.89	-0.11	-0.41	-0.01	0.07	0.09	0.08	0.03	0.03
EV	-0.71	-0.47	0.24	-0.39	0.18	-0.11	-0.16	0.02	0.01	0.06

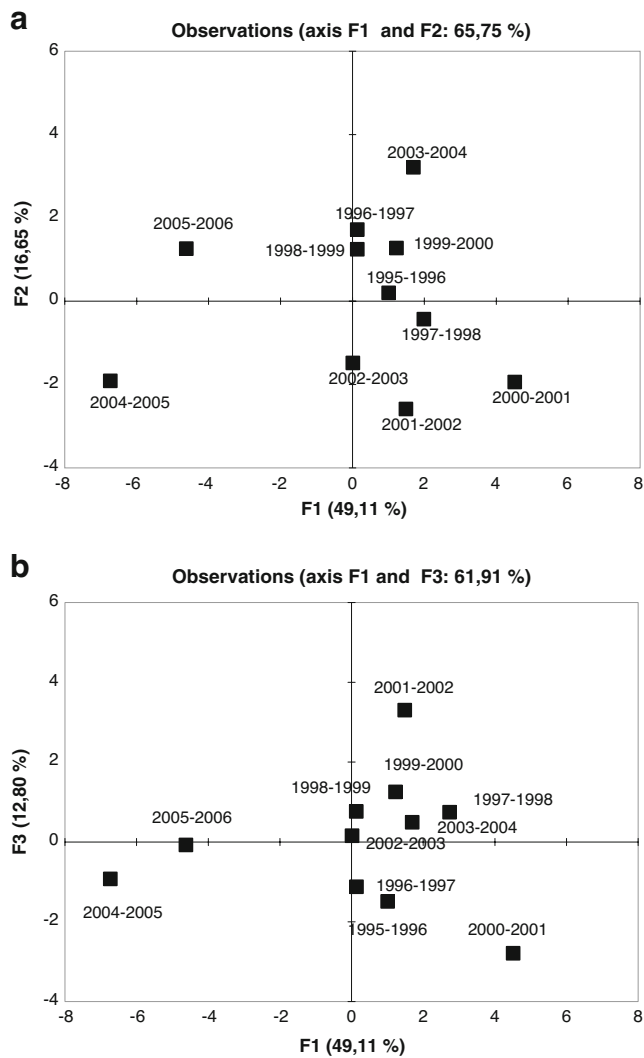


Fig. 3 a,b Principal components analysis (PCA) plots. **a** Factors 1 and 2, **b** factors 1 and 3

de Compostela (Aira et al. 1998), Lugo (Rodríguez-Rajo et al. 2003a) and Ourense (Iglesias et al. 2003).

In northern European countries such as Holland, *Alnus* pollen may account for up to 9.3% of the total (Spieksma et al. 1989), although the same authors report lower values in Perugia and Acoli Piceno (Italy). The difference may be due to an unusually strong presence of *Alnus* in the area around Leiden (Holland) (Spieksma et al. 1989). Pollen counts showed marked year-on-year variation: some authors attribute this to existence of cycles in the mobilization of nutrient reserves, although reported results are by no means consistent. Nilsson and Persson (1981) and Troise et al. (1992) observed a biannual cycle in Stockholm and in Genova, respectively, while studies by Atkinson and Larsson (1990) in Stockholm and by Rodríguez-Rajo et al. (2004) in Ourense and Santiago de Compostela, failed to identify any periodic cycle. No biannual behaviour was

observed here. Further long-term monitoring of *Alnus* pollen counts is required in order to determinate whether or not a cycle exists.

Mean *Alnus* pollen counts (1,228 pollen grains per cubic metre) in Ponferrada over the study period as a whole were similar to those reported elsewhere in northern Spain, e.g. Oviedo (Fernández Casado et al. 2002), Ourense (Iglesias et al. 1999; Méndez et al. 2002) and Vigo (Rodríguez-Rajo et al. 2002). In contrast, lower *Alnus* pollen counts have been recorded in nearby areas such as León (Fernández González et al. 1999; Vega-Maray et al. 2002) or Santiago de Compostela (Aira et al. 1999; Dopazo et al. 2002). Counts are also generally lower in southern Spain, possibly due to the relative scarcity of *Alnus* in the vegetation of these areas (Cariñanos et al. 2000; Alcazar et al. 2002; Alba et al. 2002; González Minero et al. 2002; Ruíz et al. 2002).

Higher counts have been reported in Austria and Italy than in northern European areas such as Stockholm (Atkinson and Larsson 1990; D'Amato and Spieksma 1992; Jäger et al. 1996). This may reflect differences in the climatic and geographic characteristics determining the type of vegetation in each area.

There was evidence of a delayed start to the *Alnus* pollen season. This has been described as a characteristic of winter-flowering trees, and has been compared to early flowering in spring-flowering trees; both may be a consequence of global warming (Menzel 2000; Frenguelli 2002; Rodríguez-Rajo et al. 2003a, 2004). The variation in pollen-season start dates during the study period may be due to higher temperatures during the period of chilling accumulation and low temperatures during heating accumulation (Jato et al. 2000; González-Parrado et al. 2006).

Statistical analysis

The influence of weather-related parameters, particularly temperature, on pollen counts has been highlighted by a number of authors (Frenguelli et al. 1991; Bricchi et al. 1992; Aira et al. 1998; Wielgolaski 1999; Laaidi 2001; Rodríguez-Rajo et al. 2003b; Galán et al. 2001; Jato et al. 2002b; Fornaciari et al. 2005; Emberlin et al. 2007). Here, the meteorological parameters showing the strongest correlation with airborne pollen counts in most study years were maximum and mean temperature, as also reported by other researchers (Spieksma et al. 1989; Rodríguez-Rajo et al. 2003a).

Rainfall and relative humidity had little effect in pollen counts; low correlations were also reported by Gottardini and Cristofolini (1997). Rainfall patterns tend not to be regular over the *Alnus* pollen season, and factors such as rain intensity and duration are important, although difficult to analyse (Iglesias et al. 2003); these factors may explain why rainfall had no clear effect on airborne *Alnus* pollen in

the present study. Periods with low pollen counts did not coincide with high values, since rainfall might occur at night, when pollen concentrations are low. This effect has also been pointed out by other authors (Iglesias et al. 2003).

Principal component analysis shows that the parameter exerting most influence was temperature, which formed part of factor 1, accounting for a high percentage of total variance. This finding was borne out by Spearman's correlations. Similar results have been reported by García-Mozo et al. (2008) using PCA for other taxa (*Olea europaea*). Temperature and rainfall were the parameters that best explained the distribution of pollen during the periods under study. Finally, PCA confirmed the importance of wind direction and calms. High values for calms coincided with low pollen counts.

Conclusions

A gradual and light delay in pollen-season onset was noted over the study as a whole. In most study years, the meteorological parameters displaying the strongest correlation with airborne pollen counts were maximum and mean temperature. Indeed, the highest pollen counts were recorded when the temperature range was between 9°C and 14°C. Rainfall and relative humidity had little effect on *Alnus* pollen counts. PCA showed that periods with high pollen counts were characterised by high maximum temperatures, low rainfall and an absolute humidity of around 6 g m⁻³.

Acknowledgement We would like to thank the Environmental Office of Ponferrada Municipal Government.

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