

ORIGINAL ARTICLE

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***Pinus* pollen in the atmosphere of Vigo and its relationship to meteorological factors**

Received: 17 March 1999 / Revised: 20 December 1999 / Accepted: 20 December 1999

Abstract The *Pinus* genus has an elevated pollen production and an anemophilous nature. Although considered to be hypoallergenic, numerous cases of allergies caused by *Pinus* pollen have been cited and different authors believe that its allergenicity should be studied in more depth. In the city of Vigo several patients have tested positive for *Pinus* pollen extracts in skin tests, some of them being mono-sensitive to such pollens.

In order to ascertain the behaviour of *Pinus* pollen and its correlation to the main meteorological factors, we carried out an aerobiological study in the city of Vigo from 1995 to 1998 by using a Hirst active-impact volumetric sporetrap, model Lanzoni VPPS 2000, placed on the left bank of the Vigo estuary (42°14'15"N, 8°43'30"W). *Pinus* has high quantitative importance in the airborne pollen spectrum of the city. It is one of the best represented taxa constituting 13%–20% of the total annual pollen levels. The quantity of *Pinus* pollen present in the atmosphere of the city of Vigo throughout a year is 5751 grains (as the average for the sampled years), with a very long pollination period, from the middle of January until May. The maximum concentration was recorded in 1998 with 1105 grains/m³ on 3 March, a much greater value than those for the previous years. At the end of its pollination period there is usually a final increase in *Pinus* pollen concentrations coinciding with the pollination of *Pinus silvestris*, which are more abundant in mountainous areas far from the city.

Key words Airborne pollen · Correlations · Meteorological factors · *Pinus* · Pollen concentrations · Pollinosis

Introduction

The *Pinus* genus includes a large number of species spread extensively throughout the northern hemisphere. Despite its elevated pollen production, calculated to be between 20.9×10^6 and 32.3×10^6 pollen grains per tree for *Pinus pinaster* (Tormo et al. 1996), and its anemophilous nature, it is not generally considered to be an important allergen. Different reasons are given to explain the causes of its scarce or null allergenicity: the large size of its pollen grains, which inhibits its penetration of the respiratory tract (Armentia et al. 1990), its low protein content (Cornford et al. 1990) or its hydrophobic nature due to its high lipid content (Harris and German 1985). However, pine pollen has been found in the respiratory tract: Dankaart et al. (1991) highlighted its presence in the lower respiratory tract and Accorsi et al. (1991) identified pine pollen in nasal and conjunctival secretions and, to a lesser degree, in the bronchi.

The presence of allergenic proteins in pine pollen, and their possible cross-reactivity with *Lolium perenne* allergens, have been cited by Fountain and Cornford (1990) and Cornford et al. (1990), who demonstrated a slower release of such proteins on the part of pine pollen grains. Recently, Pettyjohn and Levetin (1997) found that the quantity of protein in *Pinus echinata* pollen is higher than that of pollens such as *Juniperus ashei*, considered to be highly allergenic, linking the low allergenicity of *Pinus* to its high quantity of lipids and the slow release of allergens, since the quick release of allergens is a highly important factor in allergic reactions.

Although considered to be hypoallergenic, numerous cases of allergies caused by *Pinus* pollen have been cited (Rowe 1939; Newmark and Itkin 1967; Farnham and Vaida 1982; Harris and German 1985) and different authors believe that its allergenicity should be studied in more depth (Armentia et al. 1990; Fountain and Cornford 1991; Freeman 1993). In the city of Vigo several patients have tested positive for *Pinus* pollen extracts in skin tests, some being mono-sensitive to such pollens (Marcos et al. 1997).

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We carried out an aerobiological study of the city of Vigo from 1995 to 1998 in order to ascertain the behaviour of *Pinus* pollen and its correlation to the main meteorological factors.

Materials and methods

We placed a Hirst active-impact volumetric sporetrap, model Lanzoni VPPS 2000, on the right bank of the Vigo estuary: 42°14'15"N, 8°43'30" (Fig. 1).

We used a Nikon Ophthiphot II microscope with a 40×/0.95 resolution lens in order to identify the pollen grains. The samples were counted and prepared by the method proposed by the Spanish Aerobiology Network (Dominguez et al. 1991) and identified by means of reference guides (Valdés et al. 1987; Spieksma et al. 1993) and the palynological collection of the Ourense Aerobiology Group of the University of Vigo.

We calculated the main pollination period (MPP), following the criterion used by Nilsson and Pearson (1981), which takes into account 90% of the annual pollen concentration, eliminating the

initial 5% and the final 5%, and that used by Pathirane (1975), according to which we have taken this period to be the one described by the sigmoid curve between two inflection points.

Finally, we used Spearman's correlation coefficient to detect the possible relationship between meteorological parameters such as rainfall, humidity, hours of sunshine, temperature (maximum, mean and minimum) and wind route and direction, and the atmospheric concentration of *Pinus* pollen, studying each year separately and the 4 years of sampling together.

Results

We have collected an average of almost 6000 grains of *Pinus* pollen during the period under study (Fig. 2). The characteristics of the main pollination periods during these 4 years, according to the two criteria used, are shown in Table 1. Generally the pollination period of *Pinus* has begun by the middle of February and continues for 63 or 40 days on average, according to the aforementioned criteria. During the first 5 months of the year *Pinus* pollen is present in the atmosphere on 70%–80% of the days. Out of the taxa collected in Vigo, it represents the highest percentage in relation to the annual total (between 13% in 1995 and 21% in 1996) according to the year.

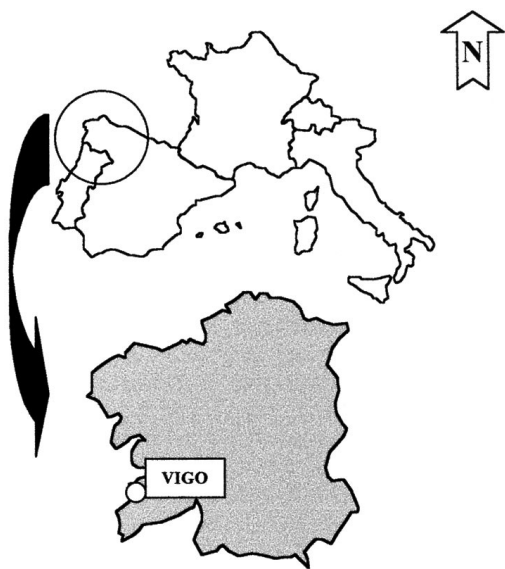


Fig. 1 Location of Vigo in Spain

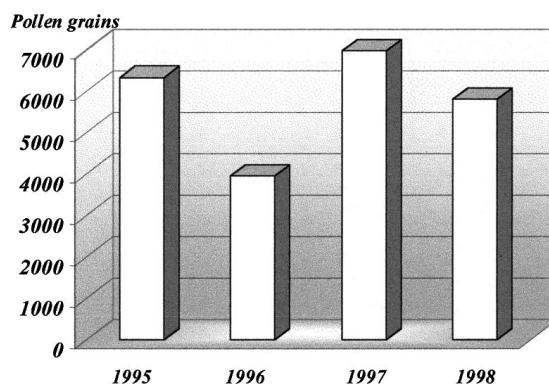


Fig. 2 *Pinus* pollen grains collected during the years under study

Table 1 Annual values of *Pinus* pollen and characteristics of its main pollination period according to Nilsson and Pearson and according to Pathirane

Characteristic	1995	1996	1997	1998
Nilsson and Pearson				
Starting date	19 February	12 March	12 February	19 February
Finishing date	20 April	6 May	9 April	7 May
Duration (days)	61	56	57	78
Average <i>Pinus</i> pollen count (grains/m ³)	94	64	112	67
Total <i>Pinus</i> pollen grains collected	6324	3955	6975	5806
Pathirane				
Starting date	10 March	21 March	3 March	19 February
Finishing date	18 April	30 April	9 April	29 March
Duration (days)	40	41	38	39
Average <i>Pinus</i> pollen count (grains/m ³)	129	80	158	132
Total <i>Pinus</i> pollen grains collected	5153	3195	5995	5144
Percentages of annual total (%)	81	81	86	89

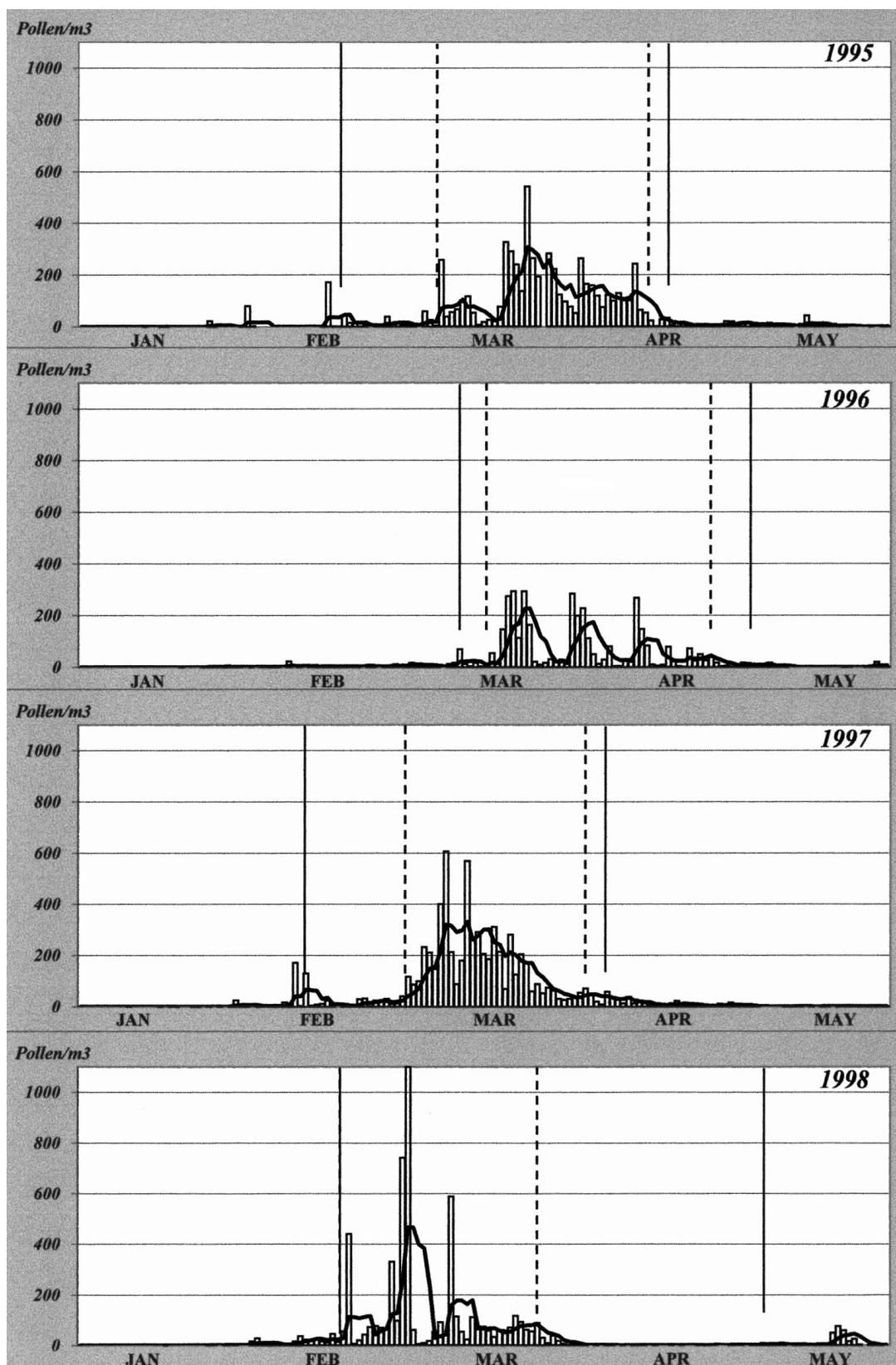


Fig. 3 Pollination period during the years under study and its running mean. Points define the period described by Pathirane and lines the one described by Nilsson and Pearson

Fig. 4 Running total of concentrations (%). Main pollination period according to Pathirane is marked

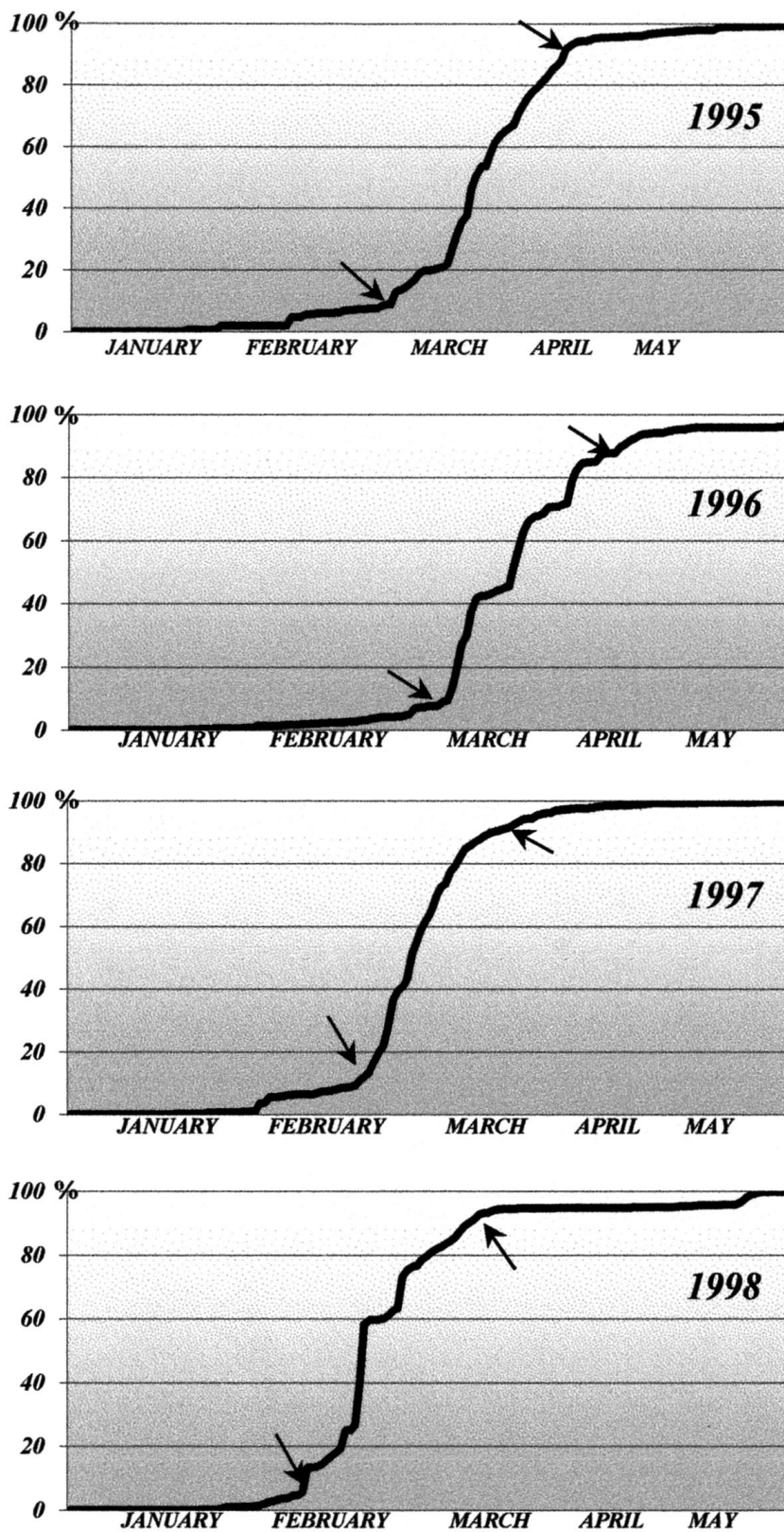


Table 2 Spearman's correlation coefficients between *Pinus* pollen concentrations and meteorological parameters, considering the main pollination period described by Nilsson and Pearson and Pathirane. T_a atmospheric temperature (maximum, minimum and median)

Period	Rainfall	Humidity	T_a max	T_a min	T_a med	Sun	Wind calm	Wind n-ne	Wind ne-s	Wind s-sw	Wind sw-n	Wind route
Nilsson and Pearson												
1995–1998	-0.430**	-0.485**	0.632**	0.186**	0.538**	0.388**	-0.201**	0.462**	0.020	-0.199**	-0.172**	-0.045
1995	-0.119	-0.427**	0.705**	0.637**	0.688**	0.526**	-0.315*	0.433**	0.467**	-0.090	-0.180	0.007
1997	-0.473**	-0.513**	0.608**	-0.498**	0.343**	0.469**	-0.296*	0.686**	-0.404**	-0.553**	-0.087	0.380**
1996	-0.159	-0.242	0.497**	0.349**	0.449**	0.023	0.073	0.112	0.006	0.138	-0.306*	-0.036
1998	-0.512**	-0.367**	0.504**	0.178	0.367**	0.231*	-0.309**	0.427**	0.161	-0.106	-0.100	-0.088
Prepeak 95–98	-0.094	-0.494**	0.649**	0.324**	0.589**	0.424**	-0.175	0.336**	0.141	-0.170	-0.134	0.138
Prepeak 95	-0.034	-0.439**	0.628**	0.587**	0.639**	0.669**	-0.228	0.331*	0.443**	-0.274	-0.380*	0.020
Prepeak 96	-0.044	-0.146	0.830**	0.841**	0.864**	-0.307	-0.209	-0.240	0.258	0.636*	-0.678*	0.316
Prepeak 97	-0.375*	-0.628**	0.703**	-0.224	0.683**	0.638**	0.003	0.445*	-0.370*	-0.361	0.334	0.217
Prepeak 98	0.009	-0.709**	0.531	0.569*	0.681**	-0.039	-0.407	-0.091	0.491	0.135	-0.641*	0.049
Pathirane												
1995–1998	-0.236**	-0.421**	0.410**	-0.060	0.239**	0.219**	-0.156*	0.353**	-0.126	-0.294**	-0.217**	0.188*
1995	-0.223	-0.256	0.566**	0.471**	0.519**	0.247	-0.246	0.377*	0.354*	-0.208	0.010	-0.062
1997	-0.183	-0.453**	0.465**	0.153	0.363*	0.111	0.054	0.131	0.086	-0.079	-0.237	-0.043
1996	-0.284	-0.165	0.260	-0.526**	-0.323*	-0.202	-0.454**	0.569**	-0.438**	-0.453**	-0.440**	0.619**
1998	-0.012	-0.432**	0.082	0.038	0.047	0.121	-0.223	0.023	0.085	-0.259	0.098	0.279
Prepeak 95–98	-0.038	-0.463**	0.630	0.438	0.615	0.334	-0.304	0.238	0.267	-0.150	-0.111	0.103
Prepeak 95	-0.298	-0.405	0.636**	0.776**	0.720**	0.775**	-0.321	0.406	0.420	-0.444*	-0.138	-0.142
Prepeak 96	-0.448	-0.648	0.684	0.540	0.720	0.445	-0.190	-0.136	-0.072	0.270	-0.208	0.540
Prepeak 97	–	-0.638*	0.781**	-0.066	0.696*	0.628*	-0.203	0.418	0.341	-0.541	0.762**	0.624*
Prepeak 98	0.009	-0.708**	0.530	0.569*	0.681**	-0.038	-0.407	-0.090	0.490	0.134	-0.640*	0.049
1995–1998	-0.236**	-0.421**	0.410**	-0.060	0.239**	0.219**	-0.156*	0.353**	-0.126	-0.294**	-0.217**	0.188*

*95% significance; **99% significance

Figure 3 shows the evolution of *Pinus* pollen during the first 5 months of the years under study and the limits of the pollination periods calculated according to the aforementioned criteria; it also shows the curves obtained when the mobile average obtained from each day and the 4 previous ones is used as a measurement of pollen concentration. One can see that the maximum values are recorded during the month of March. During this month we collected between 60% and 80% of the annual total of the *Pinus* pollen grains counted and 82% during 1996. These results can also be seen in Fig. 4, which shows the running percentage of *Pinus* pollen grains; the main pollination period according to the criterion used by Pathirane is also shown.

Finally, the Spearman correlation coefficients obtained by comparing the *Pinus* pollen concentrations and the meteorological parameters during the pollination period (Table 2) are in general, higher and constant. The correlations were calculated for both criteria, each year and all the years together; they were likewise calculated on the basis of the pollen season as a whole and the pre-peak period (Recio et al. 1997). A negative correlation is observed with precipitation, humidity and south-westerly to north-westerly winds, and a positive one with temperature, hours of sunshine and north-easterly winds.

Discussion

The concentration of *Pinus* pollen in the city of Vigo reaches very high values, the daily mean count sometimes exceeding 1000 grains/m³. Such values are generally a lot higher than those recorded in nearby parts of Galicia such as Santiago where the values do not reach 1500 grains of pollen collected from the air during the whole season (Aira et al. 1998), or Ourense, where we can only collect a total of 3000 pollen grains during the whole season (Iglesias et al. 1998).

The fact that this type of pollen includes several species means that its pollination period, based on 90% of the annual total, is long (between 64 and 112 days during the years under study) and that successive oscillations are detected in its concentrations according to the pollination of the different species. The highest concentrations occur during the pollination of *P. pinaster* (the most abundant in the city surroundings and the first to flower along with *Pinus radiata*), which means that the maximum values are recorded during the month of March, the month in which the greatest percentage, in relation to the annual total, is collected. The maximum concentration was recorded in 1998 with 1105 grains/m³ on 3 March, a much greater value than the previous years (542 in 1995, 294 in 1996 and 606 in 1997). At the end of the *P. pinaster* pollination period there is usually a final increase in pollen concentrations coinciding with the pollination of *P. silvestris*, which are more abundant in mountainous areas far from the city, so that most of the collected pollen will have been transported from more than 40 km away. In general, the concentrations collected at that

time are a lot smaller than those attained during the pollination of *P. pinaster* and *P. radiata* and are excluded when the main pollination period is calculated.

There are numerous criteria for establishing the main pollination period and the demarcation varies according to which one is chosen. Types of pollen that include different species having pollination periods that overlap or take place at different times, result in excessively long pollination periods, with various peaks throughout such periods and days in which null or very low pollen concentrations are recorded. The subsequent processing of the data, according to the chosen criterion, will include more or fewer days, thereby producing different results. When Pathirane's criterion is applied, the beginning of the period is notably delayed (except in 1998, when the beginning coincided with both criteria) and its end is brought forward (except in 1997 when they coincided), resulting in a much shorter period. The variations in the length and dates of the beginning and ending of the aforementioned pollination periods are also explained by the atmospheric washings produced by the rainfalls; these washings will influence the lengthening of the pollination period calculated by the 90% method described by Nilsson and Pearson. Thus, in 1997, at the beginning of the pollination period calculated according to their criterion, large amounts of rain fell and caused the remarkable difference between the starting date and that used for the Pathirane criterion. The same thing happened at the end of the pollination period in 1998, when the high concentration of rainfall registered during the first days of May extended the latter end of the pollination period.

In the same way, the aforementioned washings influenced the total concentrations of pollen registered during the years of study since, in 1996, because of the rainfall that took place during the pollination period, these annual sum fell to only 3955 pollen grains.

The correlations between *Pinus* pollen concentrations and meteorological parameters are, in general, higher and more constant if we consider the 90% pollination period. A negative correlation is observed with precipitation and humidity, and a positive one with temperature and hours of sunshine, when the 4 years under study are considered together. With regard to the influence of the wind, which comes from the inner part of the peninsula and reaches the city through the wide corridor, traced by the estuary, from the large masses of *Pinus* forests planted during the 1970s and dominating many of the coastal environments, the concentration is positive during the first quarter (with north-easterly winds) and negative during the third and fourth quarters (south-westerly to north-westerly), when the wind comes from the sea or from the big orographic barriers that limit the south of the city. This fact is reinforced by the occurrence of a negative correlation, or by its disappearance when the wind is calm.

Meteorology seems to be less important if we study the correlations obtained by calculating the period according to the criterion proposed by Pathirane. With the exception of minimum temperature and wind in the sec-

ond quarter, no significant correlations are obtained with the other meteorological parameters when the 4 years of study are considered together. When each year is considered independently, both the pre-peak period and the entire season, the correlations do not remain constant.

From this we can deduce that the meteorology is more important during the period of formation and ripening of the pollen grains than during the pollination process itself. Probably the morphology of the pollen grains of *Pinus* facilitates their persistence within the atmosphere once they have been freed, so the pollen values collected are not only a consequence of the dehiscence of the pollinic sacks, but also of the fact that the pollen grains remain airborne for a long time.

Conclusions

Pinus is one of the best represented taxa in the atmosphere of the city of Vigo where it constitutes between 13% and 21% of the total. Its concentrations are the most elevated in Spain, registering levels that are only matched in Barcelona (Belmonte et al. 1998). It has a very long pollen period, from February until May, and presents the highest concentrations of the year in March. At the end of its pollination period there is usually a final increase in its pollen concentrations coinciding with the pollination of *P. silvestris*.

We have found that there are five meteorological parameters that mostly influence the atmospheric concentration of *Pinus*. The positive ones are the temperature, the hours of sunshine and the wind blowing from a north, north-easterly direction, where the large forestall masses of *Pinus* are situated in the estuary of Vigo. The negative ones are rainfall and humidity, acting together and decreasing the concentrations during the pollination periods, as well as lengthening the number of days such that the differences between in the pollinic seasons, calculated according to the two criteria used, are emphasized in those years with abundant rainfall at the beginning and ending of the seasons.

Owing to the high production of pollen and to its allergenic character it is very important that, in future work, the pollination periods of the different species that form the *Pinus* family are differentiated so that its behaviour can be better understood.

Acknowledgements This work has been funded by the MAB 97-0457.C07-03 D.G.I.C.Y.T. and the XUGA 20006-B97 Xunta de Galicia projects. The authors thank the Servicio de Medio Ambiente of the Concello de Vigo for its valuable collaboration.

References

- Accorsi CA, Bandini Mazzant M, Forlani L, Rivasi F (1991) Pollen grains in human cytology. *Grana* 30:102–108
- Aira MJ, Dopazo A, Rodríguez FJ (1998) Aerobiología en Galicia: Estación de Santiago de Compostela (1997) *Bol. R.E.A.* 4:107–110
- Armentia A, Quintero A, Fernández-García A, Salvador J, Martín-Santos JM (1990) Allergy to pine pollen and pinon nuts: a review of three cases. *Ann Allergy* 64:49–53
- Belmonte J, Vendrell M, Roure JM (1998) Aerobiología en Cataluña: Estación de Barcelona (1995–1996) *Bol. R.E.A.* 3:53–56
- Cornford CA, Fountain DW, Burr RG (1990) IgE-binding proteins from pine (*Pinus radiata* D. Don) pollen: evidence for cross-reactivity with ryegrass (*Lolium perenne*) *Int Arch Allergy Appl Immunol* 93:41–60
- Dankaart WFJM, Smithius LOMJ, Blaauw PJ, Spijksma FThM (1991) The appearance of pollen in the lower airways. *Grana* 30:113–114
- Domínguez E, Galán C, Villamandos F, Infante F (1991) Manejo y evaluación de los datos obtenidos en los muestreos aerobiológicos. *Mono. R.E.A.* 1:1–18
- Farnham JE, Vaida GA (1982) A new look at a New England tree pollen. *N Engl Soc Allergy Proc* 3:320–326
- Fountain DW, Cornford CA (1991) Aerobiology and allergenicity of *Pinus radiata* pollen in New Zealand. *Grana* 30:71–75
- Freeman GL (1993) Pine pollen allergy in Northern Arizona. *Ann Allergy* 70:491–495
- Harris MC, German DF (1985) The incidence of pine pollen reactivity in an allergic atopic population. *Ann Allergy* 55:678–679
- Iglesias MI, Méndez J, Seijo MC (1998) Aerobiología en Galicia: Estación de Ourense (1997) *Bol. R.E.A.* 4:111–114
- Marcos C, Luna I, Rodríguez FJ, Jato MV, González R (1997) Pine pollen allergy. Proceedings of the EAACI'97 Rhodes (Greece)
- Newmark FM, Itkin IH (1967) Asthma due to pine pollen. *Ann Allergy* 47:251–252
- Nilsson S, Persson S (1981) Tree pollen spectra in the Stockholm region (Sweden), 1973–1980. *Grana* 20:179–182
- Pathirane L (1975) Graphical determination of the main pollen season. *Pollen Spores* 17:609–610
- Pettyjohn ME, Levetin E (1997) A comparative biochemical study of conifer pollen allergens. *Aerobiologia* 13:259–267
- Recio M, Cabezudo B, Trigo MM, Toro FJ (1997) Accumulative air temperature as a predicting parameter for daily airborne olive pollen (*Olea europaea* L.) during the pre-peak period in Málaga (Western Mediterranean area) *Grana* 36:44–48
- Rowe AH (1939) Pine pollen allergy. *J Allergy* 10:377–378
- Spijksma FTh, Nolard N, Frenguelli G, Van Moerbeke D (1993) Pollen atmosférico en Europa. U.C.B.: eds. Bruselas (Belgium)
- Tormo Molina R, Muñoz Rodríguez A, Silva Palacios I, Gallardo López F (1996) Pollen production in anemophilous trees. *Grana* 35:38–46
- Valdés B, Díez MJ, Fernández I (eds) (1987) Atlas polínico de Andalucía Occidental. Inst. de desarrollo Regional de la Universidad de Sevilla. Excma. Diputación de Cádiz eds. Sevilla (Spain)