Article

Pinus Pollen Emission Patterns in Different Bioclimatic Areas of the Iberian Peninsula

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Abstract: Background: In the Northern Hemisphere, pine forests predominate due to their natural distribution and silvicultural importance. *Pinus* tree species are large pollen producers. Nowadays, the context of climate change influences their distribution, abundance, growth and productivity. The objectives of the study were to assess the patterns of the *Pinus* flowering behavior regarding their pollen presence in the atmosphere and intensity in different bioclimatic areas of the Iberian Peninsula during recent years. Methods: The survey was carried out in three different biogeographic zones of Spain: Vigo (Eurosiberian region) and Ourense (transition area between the Eurosiberian and Mediterranean areas) located in northwest Spain and Toledo (Mediterranean area) placed in the center of the Iberian Peninsula. Airborne pollen was collected with volumetric traps in each study area from 1995 to 2019. Results: *Pinus* pollen showed a marked single pollination period during late March in the Eurosiberian region and April in the transition zone between the Eurosiberian and the Mediterranean area. Two different peaks with lower pollen intensity were detected during the pollen season in Toledo (Mediterranean area), the first during late March and the second from the end of May to the beginning of June. The trends detected revealed changes in the timing of the phenological cycle, such as longer pollen seasons and later end dates of the Main Pollen Season (MPS) in some cases. The mean Annual Pollen Integral (API) in the Eurosiberian area (Vigo) and transition zone (Ourense) was similar, with about 4400 pollen grains. In the Mediterranean area (Toledo), a lower API amount of 1618 pollen grains was recorded. A trend towards an increase of 126 and 80 pollen grains per year in the airborne pine load was detected in the transition and Mediterranean areas studied, respectively. Conclusions: The rates of the annual integral *Pinus* pollen percentage with respect to the total pollen of forest species in the atmosphere of the areas studied showed a decreasing percentage trend during the last years.

Keywords: pine forests; aerobiology; airborne pollen; pollination period; meteorological variables; trends

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1. Introduction

The *Pinus* genus is very important from an ecological point of view in the Northern Hemisphere as its species are often dominant in forests [1] with a high representativeness in the pollen spectra of some regions [2]. In the south of Europe, numerous pine species naturally grow, forming mixed and pure coniferous forests [3]. In Mediterranean areas, pine species have been greatly influenced by humans who favored the extension of the forests for native species and included new allochthonous pine species in forest plantations [4].

In the Iberian Peninsula, pine forests together with oaks and holm oaks occupy most of the forest area. Native pine species are represented by *P. halepensis* Mill., *P. pinaster* Aiton, *P. pinea* L., *P. nigra* J.F. Arnold subsp. *salzmanii* (Dunal) Franco, *P. sylvestris* L. and *P. uncinata*

Ram. In northwest Spain, the main pine forests are represented for *P. pinaster*, *P. sylvestris* and the introduced *P. radiata* D. Don, while, in the central part of the country, the main species are *P. pinaster*, *P. sylvestris*, *P. pinea*, *P. nigra* subsp. *salzmanii* and *P. halepensis* [5]. The real context of climatic change influences their distribution, abundance, growth and productivity during recent years [6]. The main effects of climatic change will be visible in Southern Europe in 2080, as the worst projection scenario has predicted an increase in the average annual temperature of 4–6 °C and a decrease in average annual precipitation of 15–20% [7]. These environmental changes will influence the expansion or restriction in the distribution area of some species [8,9]. In particular, forest is especially sensitive to climate change, since trees have a long lifespan, and this condition does not allow these plants to adapt quickly to environmental changes [10]. In addition, forest species are more vulnerable to climate change during the juvenile and reproductive phases, since the root system is not yet fully developed for young plants, and, during the reproductive phase, forest species need favorable climatic conditions to produce more seeds [11]. Other factors, such as fires, diseases, changes in the atmospheric composition [12] or anthropogenic activities, also have important impacts on biological systems [13].

Some investigations identified the *Pinus* pollen as one of the most abundant airborne forest pollen taxa in the atmosphere of some regions of the Iberian Peninsula during spring or early summer [2,14,15]. Together with *Quercus* pollen, they constitute the main pollens coming from arboreal species associated with non-riparian forests, and thus have high ecological value for monitoring [16]. Pine forests produce a higher quantity of pollen than other plants such as grasses or herbs, reaching values of 100–1000 kg/ha [17]. All the Southern Europe *Pinus* species are recognized as large pollen producers, estimated as between 20.9 and 32.3 million pollen grains per tree in the case of *P. pinaster* [18].

The knowledge of airborne pollen in a given area is a useful tool to obtain information on the phenology and distribution of plants [19–21], crop production [22–24] and conservation of biodiversity [16,25,26]. Furthermore, airborne pollen data and their trends can be used as accurate indicators of environmental changes [27–30].

The main goals of the study were to study the patterns of the *Pinus* pollen production and flowering behavior in different bioclimatic areas of the Iberian Peninsula and analyze the long-term trends in pollen timing and intensity. The climatic variables that influence long-term changes of airborne pollen and their trends were also assessed, since *Pinus* pollen plays an important ecological role in native forest ecosystems and human-managed forest stands.

2. Materials and Methods

2.1. Characterization and Locations of Study Areas

The study was carried out in different bioclimatic and biogeographic areas of Spain: Vigo (Eurosiberian biogeographic region) and Ourense (transition area between the Eurosiberian and Mediterranean areas) located in northwest Spain and Toledo (Mediterranean biogeographic region) placed in the central area of the Iberian Peninsula (Figure 1). The city of Vigo (42°14' N; 8°44' W) is located on the southern bank of an estuary on the Atlantic coast; its climate is temperate maritime, although with some Mediterranean aspects, with an average annual temperature of 14 °C and annual total rainfall of 1791 mm [31]. Ourense (42°20' N; 7°52' W) is located in a depression at 139 m above sea level with a climate of greater Mediterranean influence represented by warm temperatures and low humidity, with an average annual temperature of 14.9 °C and annual total rainfall of 811 mm [31]. Toledo (39°51' N; 4°02' W) is located at 450 m above sea level in central Spain with a warmer and drier climate characterized by an average annual temperature of 15.8 °C and annual total rainfall of 342 mm [31].

Figure 1. Location of the areas studied.

2.2. Airborne Pollen Study

Airborne pollen was collected using Lanzoni VPPS-2000 volumetric traps [32], from 1995 to 2019 in Vigo and Ourense (Northwest Iberian Peninsula) and from 2003 to 2019 in Toledo (central Iberian Peninsula). The samples obtained were processed following the standardized methodology of the Spanish Aerobiological Network [33]. Pollen data were expressed as pollen grains per cubic meter of air for the Annual Pollen Integral (API) and the daily pollen concentrations [34]. To calculate the Main Pollen Season (MPS) parameters, we used the AeRobiology software package [35]. The MPS was calculated using a pollen emission model that fits a nonlinear logistic regression model to the values of the accumulated sum of daily airborne pollen concentration sampled over a year [36,37].

2.3. Meteorological Data

The meteorological data, namely maximum, minimum and average temperatures ($^{\circ}\text{C}$), rainfall (mm) and relative humidity (%), were continuously monitored by the meteorological stations of the Galician Institute for Meteorology and Oceanography METEOGALICIA “Ourense” and “Vigo” placed in both areas at 300 m from the pollen samplers. In the city of Toledo, the weather data were provided by the Spanish Meteorological Agency (AEMET) located 2 km from the pollen trap.

2.4. Statistical Analysis

To estimate the increasing or decreasing trends of the aerobiological and meteorological parameters, we performed linear regression analysis. Spearman correlation analyses were conducted between the MPS characteristics (start, end and length dates of the MPS, pollen integral, pollen peak concentration and pollen peak day) and the meteorological variables (maximum, minimum and average temperatures, rainfall and relative humidity) to test the statistical dependence. For the statistical analyses, IBM SPSS Statistics 24.0 software was applied.

3. Results

Pinus airborne pollen was recorded in the atmosphere of Vigo (Eurosiberian area) from Week 4 to Week 26 (late January–late June) of the year (Figure 2), two weeks later in the transition area of Ourense from Week 7 to Week 28 (mid-February–early July) and in the Mediterranean area between Week 11 and Week 26 (mid-March–late June).

Figure 2. Weekly distribution of *Pine* pollen during the study years in each area studied (Eurosiberian, Vigo; transition, Ourense; and Mediterranean, Toledo).

The main pollen concentrations were observed in the atmosphere of northwestern Spain during the spring months. In the Eurosiberian study area of Vigo, the MPS began on average on 25 February and ended two months later on 24 April (following the logistic method for the definition of the pollen season), registering a similar relative standard deviation (around 18%) for the start and end dates. The earliest start of the MPS occurred on 2 January 2001 and the latest on 27 March 2018. The earliest date of the end of the MPS was observed on 24 March 2000 and the latest on 12 June 2013. The average duration of the MPS was 60 days, with a range from 22 days in 2000 to 138 days in 2001. The average API was 4420 pollen grains/m³ with a maximum pollen peak (1222 pollen grains/m³) on 26 March 2002 (Table 1).

Table 1. *Pinus* MPS during the 25 study years in the Eurosiberian area (Vigo): start date, end date, length, API (pollen grains), daily pollen peak (pollen grains/m³) and peak date. Average, minimum and maximum values, standard deviation (SD) and relative standard variation in percentage (%RSD) are included. The logistic method was used.

MPS—Vigo						
Year	Start (MPS)	End (MPS)	Length (MPS)	API	Pollen Peak	Peak Day
1995	28-Feb.	23-Apr.	55	5616	542	25-Mar.
1996	3-Mar.	30-Apr.	59	3588	294	22-Mar.
1997	21-Feb.	4-Apr.	43	5993	606	10-Mar.

Table 1. Cont.

MPS—Vigo						
Year	Start (MPS)	End (MPS)	Length (MPS)	API	Pollen Peak	Peak Day
1998	11-Feb.	27-Mar.	45	5297	1105	3-Mar.
1999	28-Feb.	17-Apr.	49	4080	489	7-Apr.
2000	3-Mar.	24-Mar.	22	7704	1131	11-Mar.
2001	2-Jan.	19-May	138	1529	408	5-Mar.
2002	3-Mar.	15-Apr.	44	6894	1222	26-Mar.
2003	4-Mar.	8-Apr.	36	5598	989	17-Mar.
2004	10-Feb.	1-May	82	4749	351	3-Mar.
2005	14-Mar.	17-Apr.	35	3343	1129	31-Mar.
2006	13-Mar.	8-May	57	4962	863	3-Apr.
2007	26-Feb.	7-May	71	5488	374	17-Apr.
2008	5-Feb.	24-Apr.	80	2158	185	19-Mar.
2009	27-Feb.	12-Apr.	45	4004	347	17-Mar.
2010	15-Mar.	6-May	53	2951	321	11-Apr.
2011	1-Mar.	27-Apr.	58	4469	576	1-Apr.
2012	5-Mar.	8-Apr.	35	4338	575	23-Mar.
2013	27-Jan.	12-Jun.	137	2365	167	18-Apr.
2014	4-Mar.	6-May	64	3042	323	9-Apr.
2015	19-Mar.	21-Apr.	34	8780	1027	8-Apr.
2016	12-Feb.	18-May	97	2617	220	21-Mar.
2017	25-Feb.	22-Apr.	57	5734	844	25-Mar.
2018	27-Mar.	21-May	56	3657	522	25-Apr.
2019	27-Feb.	15-Apr.	48	1538	147	26-Mar.
Mean	25-Feb.	24-Apr.	60	4420	590	26-Mar.
Max	27-Mar.	12-Jun.	138	8780	1222	26-Mar.
Min	2-Jan.	24-Mar.	22	1529	147	26-Mar.
SD	17.519	18.353	28.666	1840.404	345.096	14.325
RSD%	31.019	15.893	47.777	41.641	58.463	16.712

The mean MPS began in the transition area of Ourense on 13 March and ended on 30 April, with a relative standard deviation around 12%. The year with the earliest start date of the MPS was 1998 (22 February) and the latest in 2018 (17 April). The earliest end date was 7 April 1997 and the latest one was recorded on 31 May 2016. The average duration of the MPS was 50 days, ranging from 26 days in 2003 to 99 days in 2016. The average API was 4405 pollen grains/m³ with a maximum pollen peak of 1003 pollen grains/m³ recorded on 25 March 2002 (Table 2).

Table 2. *Pinus* MPS during the 25 study years in the transition Eurosiberian (Ourense): start date, end date, length, API (pollen grains), daily pollen peak (pollen grains/m³) and peak date. Average, minimum and maximum values, standard deviation (SD) and relative standard variation in percentage (%RSD) are included. The logistic method was used.

MPS—Ourense						
Year	Start (MPS)	End (MPS)	Length (MPS)	API	Pollen Peak	Peak Day
1995	6-Mar.	22-Apr.	48	3696	627	25-Mar.
1996	22-Mar.	9-May	49	3505	442	9-Apr.
1997	4-Mar.	7-Apr.	35	2422	287	18-Mar.
1998	22-Feb.	21-Apr.	59	3114	292	22-Mar.
1999	20-Mar.	21-Apr.	33	1694	323	6-Apr.
2000	3-Mar.	19-Apr.	48	2600	233	27-Mar.

Table 2. Cont.

MPS—Ourense						
Year	Start (MPS)	End (MPS)	Length (MPS)	API	Pollen Peak	Peak Day
2001	23-Feb.	3-May	70	1778	290	1-Apr.
2002	11-Mar.	24-Apr.	45	6262	1003	25-Mar.
2003	19-Mar.	13-Apr.	26	3369	818	1-Apr.
2004	2-Mar.	3-May	63	3294	314	4-Apr.
2005	24-Mar.	22-Apr.	30	2851	656	7-Apr.
2006	31-Mar.	7-May	38	5615	728	10-Apr.
2007	11-Mar.	8-May	59	5814	287	16-Apr.
2008	13-Mar.	26-Apr.	45	3690	552	6-Apr.
2009	5-Mar.	22-Apr.	49	5986	464	24-Mar.
2010	2-Apr.	8-May	37	5532	644	12-Apr.
2011	18-Mar.	28-Apr.	42	5494	649	11-Apr.
2012	12-Mar.	15-Apr.	35	5507	565	30-Mar.
2013	25-Mar.	14-May	51	4552	285	17-Apr.
2014	15-Mar.	3-May	50	7990	899	10-Apr.
2015	11-Mar.	5-May	56	6783	548	3-Apr.
2016	23-Feb.	31-May	99	5250	278	28-Feb.
2017	9-Mar.	6-May	59	5576	295	8-Apr.
2018	7-Apr.	17-May	41	6095	604	27-Apr.
2019	24-Feb.	10-May	76	1660	178	31-Mar.
Mean	13-Mar.	30-Apr.	50	4405	490	3-Apr.
Max	7-Apr.	31-May	99	7990	1003	25-Mar.
Min	22-Feb.	7-Apr.	26	1660	178	31-Mar.
SD	12.123	12.066	16.017	1833.928	226.165	11.394
RSD%	16.913	10.021	32.214	40.992	46.117	12.241

In the Mediterranean area of Toledo, the *Pinus* pollen MPS occurred during spring and early summer. The average start date during the study period was registered on 6 March and the end date on 29 June, with relative standard deviations of around 13% and 15%, respectively. The earliest start date was observed on 18 February 2008, while 2015 registered the latest date (11 April). The earliest MPS end date was on 2 June 2017 and the latest one occurred on 25 July in both 2016 and 2018. The average duration of the MPS was 116 days, with a range from 74 days in 2015 to 154 days in 2016. The average API was 1618 pollen grains/m³ with a pollen peak of 503 pollen grains/m³ on 1 April 2014 (Table 3).

Table 3. *Pinus* MPS during the 25 study years in the Mediterranean area (Toledo): start date, end date, length, API (pollen grains), daily pollen peak (pollen grains/m³) and peak date. Average, minimum and maximum values, standard deviation (SD) and relative standard variation in percentage (%RSD) are included. The logistic method was used.

MPS—Toledo						
Year	Start (MPS)	End (MPS)	Length (MPS)	API	Pollen Peak	Peak Day
2003	24-Feb.	10-Jun.	107	781	43	22-Mar.
2004	22-Feb.	15-Jul.	145	1092	98	31-Mar.
2005	15-Mar.	6-Jul.	114	1491	89	5-Jun.
2006	8-Mar.	14-Jun.	99	788	81	4-Apr.
2007	4-Mar.	1-Jul.	120	1633	127	30-Mar.
2008	18-Feb.	15-Jun.	119	1755	235	30-Mar.
2009	3-Mar.	30-Jun.	120	1629	117	27-May

Table 3. Cont.

MPS—Toledo						
Year	Start (MPS)	End (MPS)	Length (MPS)	API	Pollen Peak	Peak Day
2010	20-Mar.	11-Jul.	114	1332	81	4-Jun.
2011	5-Mar.	20-Jun.	108	1039	152	3-Apr.
2012	5-Mar.	21-Jun.	109	1332	70	10-Apr.
2013	5-Mar.	3-Jul.	121	1273	103	11-Apr.
2014	27-Feb.	23-Jun.	117	2695	503	1-Apr.
2015	11-Apr.	23-Jun.	74	2654	339	20-May
2016	23-Feb.	25-Jul.	154	1775	148	3-Apr.
2017	21-Feb.	2-Jun.	102	1615	155	26-Mar.
2018	22-Mar.	25-Jul.	126	2390	178	17-Jun.
2019	8-Mar.	7-Jul.	122	2228	131	28-May
Mean	6-Mar.	29-Jun.	116	1618	156	23-Apr.
Max	11-Apr.	25-Jul.	154	2695	503	1-Apr.
Min	18-Feb.	2-Jun.	74	781	43	22-Mar.
SD	12.967	15.120	17.569	588.611	113.404	30.658
RSD%	19.896	8.394	15.154	36.385	72.750	27.089

Pinus pollen represented on average around 28–41% of the total annual pollen of forest tree species registered in the atmosphere of Vigo and Ourense, respectively. Figure 3 shows a negative trend in the percentage of *Pinus* pollen registered in the atmosphere over the year with respect to the total airborne pollen of forest tree species present, being more pronounced in Vigo (decrease of 1.498% of relative abundance by year) than in Ourense (decrease of 0.731% by year). This reduction may indicate that the *Pinus* forests in both study areas are decreasing and being replaced by other taxa, such as *Quercus*, which showed an increase of around 0.7% of relative abundance in the total pollen of trees registered in the atmosphere of the Eurosiberian and transition areas (Figure 3). In the case of Toledo (Mediterranean area), there is no significant trend for either *Pinus* or *Quercus*.

To elucidate the meteorological parameters that influence the seasonal dynamics and intensity of the airborne pine pollen, a Spearman correlation study was carried out with the average values of the meteorological variables registered during the MPS and the previous month in the years of the study (Table 4). The start date of the MPS was synchronized with the main meteorological parameters, since water-related variables (rainfall and relative humidity) were negatively correlated with the MPS start date in the Eurosiberian and transition areas, while they were positively correlated in the Mediterranean area. Temperatures were positively correlated with the MPS start date in the Eurosiberian region. The end of the MPS was delayed as a consequence of higher values of the variables related to water (rainfall and relative humidity) in the Eurosiberian region and the minimum temperature in the transition and Mediterranean areas. The length of the MPS increased with higher rainfall and minimum temperature, depending on the bioclimatic area. The API was positively influenced by rainfall and maximum temperatures in the Mediterranean area, whereas it was negatively influenced by the water-related parameters (rainfall and relative humidity) in the Eurosiberian area and the rainfall and minimum temperatures in the transition area. The peak dates were delayed as a consequence of higher minimum temperatures in the Eurosiberian area and high values of the water-related parameters in the Mediterranean area.

Figure 3. Trend of the percentage of airborne *Pinus* pollen concentration compared to the total forest tree species airborne pollen content during the entire study period in each study area.

In addition, a regression study was carried out to elucidate the trends observed in recent years (Table 5 and Figure 4). Considering the weather variables during the MPS, we obtained significant trends in the Eurosiberian bioclimatic area for minimum and average temperatures with increases of 0.169 and 0.078 °C per year, respectively. The same behavior was observed over the pollen pre-peak period for the minimum temperatures and over the post-peak period for the average temperature (Table 5). Significant statistical trends were obtained in the Eurosiberian region for the end of the MPS and the pollen peak date, since the average dates happened in both cases 0.994 days later per year (Figure 4). In the transition zone between the Eurosiberian and Mediterranean areas, a significant trend was detected towards a delay in the end of the MPS of 0.961 days per year, as well as an increase in the API of 126 pollen grains per year (Figure 4). Considering the meteorological variables, we detected a significant and positive increased trend with the minimum temperatures

during the post-peak period. Finally, in the Mediterranean area, an increasing trend in the API of 80 pollen grains per year was found.

Table 4. Spearman correlations between the MPS characteristics (start, end and length dates of the MPS, API and pollen peak day) and the meteorological variables (maximum, minimum and average temperatures, total rainfall and relative humidity). Significance level was represented as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Area	MaxT	MinT	AvgT	Rainfall	Humidity
MPS Start date	Eurosiberian (Vigo)	0.406 **	0.437 **	0.489 **	−0.721 ***	−0.383 *
	Transition (Orense)				−0.467 **	−0.340 *
	Mediterranean (Toledo)	0.787 ***		−0.430 *	0.731 ***	0.827 ***
MPS End date	Eurosiberian (Vigo)				0.561 ***	0.427 **
	Transition (Orense)		0.592 ***			
	Mediterranean (Toledo)		0.472 *			
MPS Length	Eurosiberian (Vigo)	−0.375 *			0.801 ***	0.509 ***
	Transition (Orense)	−0.337 *	0.454 **		0.414 **	
	Mediterranean (Toledo)		0.581 **			
API	Eurosiberian (Vigo)				−0.532 ***	−0.340 *
	Transition (Orense)		−0.369 *		−0.451 **	
	Mediterranean (Toledo)	0.444 *		−0.473 *	0.469 *	
Peak date	Eurosiberian (Vigo)		0.616 **	0.458 **		
	Transition (Orense)		0.364 *			
	Mediterranean (Toledo)	0.757 **		−0.546 **	0.718 **	0.778 **

Table 5. Trends of the MPS characteristics (start, end and length dates of the MPS, pollen integral, pollen peak concentration and pollen peak day) and meteorological variables (maximum, minimum and average temperatures, total rainfall and relative humidity). The shown parameters of the linear regression analysis are slope, R^2 value and p -value. The significant results are shown in bold.

		Eurosiberian Vigo (1995–2019)			Transition Ourense (1995–2019)			Mediterranean Toledo (2003–2019)		
		Slope	R^2	p	Slope	R^2	p	Slope	R^2	p
MPS	Start MPS	0.493	0.043	0.331	0.265	0.026	0.462	0.645	0.063	0.329
	End MPS	0.994	0.158	0.049	0.961	0.346	0.002	0.649	0.048	0.410
	Length MPS	0.506	0.017	0.536	0.702	0.104	0.116	−0.007	0.000	0.994
	Pollen integral	−68.523	0.075	0.185	126.130	0.256	0.009	79.717	0.468	0.002
	Pollen peak	−13.718	0.085	0.156	0.270	0.000	0.967	8.843	0.155	0.118
	Peak date	0.994	0.255	0.009	0.391	0.063	0.228	1.750	0.082	0.265
Year	Max T	0.022	0.009	0.650	0.003	0.000	0.958	0.173	0.176	0.093
	Min T	0.169	0.491	0.000	0.037	0.039	0.343	0.106	0.119	0.175
	Average T	0.078	0.169	0.041	−0.013	0.009	0.647	0.139	0.156	0.116
	Rainfall	−0.741	0.000	0.931	1.632	0.018	0.520	−2.922	0.043	0.422
	Rel Hum	0.067	0.012	0.608	−0.173	0.102	0.118	−0.464	0.185	0.085

Table 5. Cont.

		Eurosiberian Vigo (1995–2019)			Transition Ourense (1995–2019)			Mediterranean Toledo (2003–2019)		
		Slope	R ²	p	Slope	R ²	p	Slope	R ²	p
Pre-peak Period	Max T	0.016	0.004	0.766	−0.040	0.012	0.594	0.133	0.044	0.419
	Min T	0.151	0.322	0.003	−0.040	0.024	0.456	0.095	0.038	0.454
	Average T	0.067	0.085	0.157	−0.075	0.129	0.077	0.114	0.044	0.148
	Rainfall	1.646	0.006	0.715	−0.588	0.005	0.734	1582	0.033	0.486
	Rel Hum	0.010	0.000	0.950	−0.227	0.073	0.192	−0.121	0.006	0.761
Post-peak Period	Max T	0.042	0.023	0.469	0.016	0.002	0.831	0.307	0.152	0.121
	Min T	0.202	0.493	0.702	0.009	0.172	0.039	0.194	0.094	0.231
	Average T	0.103	0.219	0.018	0.027	0.0153	0.556	0.250	0.125	0.163
	Rainfall	−2.388	0.012	0.602	2.220	0.050	0.281	−4504	0.139	0.140
	Rel Hum	0.132	0.032	0.393	−0.113	0.037	0.356	−0.914	0.265	0.034

Figure 4. Significant trends of the airborne *Pinus* main pollen season (MPS) during the entire study period in each study area.

Finally, the correlations between the API and rainfall during the MPS in all years in the three bioclimatic areas of the study showed a negative relationship, with the highest

Spearman coefficient observed in Vigo (0.504 $p < 0.01$). The years with less precipitation produced more pollen (Figure 5).

Figure 5. The relationship between airborne *Pinus* pollen and rainfall during the main pollen season (MPS) of all study years in each study area.

4. Discussion

During the last decades, several studies have investigated the vulnerability of forests to pressures related to the environmental impacts of global climate change [38,39], pests and diseases [40,41], wildfires or anthropological actions such as changes in land use [42] and pollution [43]. Aerobiological variables represent a useful tool to evaluate the variation in the seasonal dynamics of these ecosystems with multiple applications [16,44,45].

Pinus forests cover an extensive area on the Northern Hemisphere, often being the predominant species in boreal, subalpine, temperate and even arid conditions [1,46]. Pines belong to the natural Mediterranean vegetation [3], but this genus was profoundly favored and used as a key tree for land afforestation during the first half of the 20th century throughout the Eurosiberian and Mediterranean areas [4,47]. The choice of pine species in afforestation programs is the results of their suitability for timber, resin and food industries, as well as their fast growth and resistance for the recovery of deforested or fire-damaged areas, where they act as pioneer species [48]. Currently, pine forests represent around 28% of the area occupied by wooded forest formations in Spain [5]. However, the distribution throughout the Iberian Peninsula is very irregular, not only following natural areas with optimal environmental characteristics for pine forests, but also because of the different afforestation efforts in different regions using different pine species depending on the area [49]. Plantations using the native *P. pinaster* and the allochthonous *P. radiata* have been extended in the northwest areas of the Iberian Peninsula (surroundings of Vigo and Ourense). In the vicinity of the city of Toledo, however, pine afforestation is less common, although the native *P. halepensis* and *P. pinea* have been often used in parks and recreational areas. Therefore, the human influence on the *Pinus* pollen concentrations is very relevant.

Pinus pollen showed a marked single pollination period (one main pollen peak) during late March in the Eurosiberian region and the month of April in the transition zone between the Eurosiberian and the Mediterranean area. Otherwise, two pollination periods were detected in the Mediterranean area: the first period recorded from mid-March to the early April and the second period recorded between the late May to the early June. The distribution of the species of the genus and the differences on the timing of their flowering periods in each study area can explain these differences. Several investigations have indicated that the phenology and MPS of different pine species are regulated by their sensitivity to biometeorological and weather conditions [50,51]. For example, *P. halepensis*, *P. pinea* and *P. pinaster* show less sensitivity to winter temperatures and better withstand the summer drought in the first months of the year [50,51]. Other species such as *P. halepensis* shows relationships with mid-winter temperatures and intermediate annual rainfall values [52]. These differences between species can explain the temporal variations in the MPS among the three bioclimatic areas, since the flowering period takes place first in Vigo, then in Ourense and later in Toledo. However, this phenological pattern may also be due to the altitudinal gradient from northwestern to central areas of the Iberian Peninsula (from 0 to 450 m above sea level) [53].

Temperature is the meteorological variable with the greatest influence on the synchronization of ecological processes [2,28,54,55]. Not only are changes observed at shorter time scales, but longer-term trends in climate also impact biological processes [56]. Climate change has already been shown to be an important factor in timing and intensity of pollination [21,29]. Our study detected a statistically significant positive trend in minimum and average temperatures throughout all study years in the Eurosiberian region. In addition, a decrease in relative humidity was detected in the Mediterranean study area throughout the year. These weather variations can induce changes in the phenological cycle of the pine genus members, such as a longer pollen season. In the case of the Eurosiberian and the transition areas, a significant trend was detected towards the delay of the end of the MPS of around one day per year. Similar results have been observed in other studies conducted across Europe [2,57].

The average API in the Eurosiberian and transition areas studied was similar, with around 4400 pollen grains. A trend towards an increase of 126 and 80 pollen grains per

year in the airborne pollen load of pine was detected in Ourense (transition site) and Toledo (Mediterranean site), respectively, according to the findings noted by several authors [27,58]. In the Eurosiberian area, a not significant decrease in the trend of the API was detected, as a consequence of the expansion in the area of invasive species of pine tree pests such as *Leptoglossus occidentalis* (Hemiptera, Coreidae) [59]. In the Mediterranean area studied, a lower API amount of 1618 pollen grains was registered; these results agree with the data presented by De Linares et al. [2]. The high *Pinus* pollen concentrations recorded in northwestern Spain induces important ecological modifications in the timings of other bio-ecosystems, increasing their primary productivity in the non-favorable season of the year [60–62]. Pollen grains decompose rapidly in aquatic and terrestrial ecosystems, releasing large amounts of nutrient-rich matter [63–66]. Several authors pointed out that input pulses of individual elements by pine pollen rains can reach 0.3–0.5 kg/ha N, 0.04–0.07 kg/ha P, 0.1–0.2 kg/ha K, 0.02 kg/ha S and 0.01 kg/ha Mg [64,67,68] during the atmospheric pine pollen season. Terrestrial detritivores and fungi are some of the most benefited organisms, providing ecosystems with other additional limiting elements such as C:N:P, and especially K, S and Cu [62]. Maggs [69] showed that, although pollen grains only constitute 3.5% of the total annual biomass of pine litter for decomposition, they represent more than 30% of the total amount of N, P and K deposited in their associated bio-ecosystems. Thus, intensive pollen rains can temporally mitigate limiting nutritional periods for primary production experienced in terrestrial and aquatic ecosystems [62]. The highest concentrations of pine pollen recorded in the Eurosiberian area of northwestern Spain can act as a temporary pulse of nutrients during early spring for their important maritime fisheries for fish and shellfish. Spring pollen rains dominated by anemophilic trees have been shown to move considerable amounts of nutrients from terrestrial to aquatic ecosystems [60,66] when young detritivores develop and have their largest nutritional requirements [70]. This fact allows the proliferation of microalgae in early spring, increasing the primary productivity rates of the maritime area [60] in the Eurosiberian zone of northwestern Spain.

Pine pollen grains represent 22–32% of the annual total of tree pollen in the atmosphere of northwestern Spain, even though *Pinus* spp. forests only represent 17–25% of the forest areas in the provinces studied [5]. Likewise, the National Forest Inventory of Spain registered that *Pinus* spp. forests represent 3% of the total area of forests, while 8% of the tree pollen grains registered during the year in the atmosphere belongs to the genus *Pinus* [5]. When we analyze these rates of *Pinus* pollen percentage with respect to the total pollen of the forest species in the areas studied, a decreasing percentage trend was detected in recent years in the northwest of the Iberian Peninsula. This tendency could indicate a restoration of natural oak forests in the Eurosiberian area as a sign of ecological maturity of the vegetation distribution [71–74]. Previous studies in the Eurosiberian and transition areas observed a trend of continuous growth of autochthonous oak forest during the last two decades [74–76]. The changes were mainly due to variations in land use through reforestation or the natural regeneration of abandoned agricultural lands. On the contrary, natural oak forests have been replaced by dry olive groves, reducing their representation in the atmosphere in some Mediterranean areas [77] and resulting in low percentages of pine forest areas compared to the total surface of tree forests.

Pollen production and dispersal are more strongly modulated by water-related climate variables. Our study shows that rainy years registered a lower API, and vice versa for dry years. This behavior was pointed out by several authors in different areas [73,78–81]. Furthermore, relative humidity during the MPS showed a significant negative correlation with pollen concentrations. The behavior of these two variables could indicate that pollen absorbs humidity from the air, which induces an increase in the weight of the pollen and a deposition closer to the pollinating tree.

5. Conclusions

Pinus pollen showed a marked single pollination period during late March in the Eurosiberian region or in April in the transition zone between the Eurosiberian and Mediterranean areas. Two pollination periods were observed in the Mediterranean area, the first recorded at the end of March and the second between late May and the beginning of June. The trends detected in the meteorological variables for the three study areas induced changes in the phenological cycle of the pine genus, such as longer pollen seasons and delays in the end of the MPS. The mean API in the studied Eurosiberian and transition areas was 2–3 times higher than the one recorded in the Mediterranean area studied. A trend towards an increase of 126 and 80 pollen grains per year in the atmospheric load of pine was detected in the transition and Mediterranean areas studied, respectively. The annual percentage of the *Pinus* integral with respect to the total pollen of forest species in the areas studied showed a decreasing trend during recent years. This tendency could indicate a restoration of natural oak forests in the Eurosiberian area as a sign of ecological maturity of the vegetation distribution.

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