TITLE PAGE

2	Title:	Pollen	season	trends	in	winter.	flou	vering	trees	in	South Spai	n
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4 Abstract.

The present work has studied the trends in pollen seasons of winter flowering trees (Alnus, 5 Cupressaceae, Fraxinus, Populus and Ulmus) in Córdoba, Granada and Málaga 6 (Andalusian, Spain) over the years 1994-2017. The influence of meteorological 7 parameters in the seasonal airborne pollen has been also analyzed. Pollen concentrations 8 9 were recorded using Hirst-type volumetric spore traps, following the standardized methodology of the Spanish Aerobiology Network (REA) and the European Aerobiology 10 Society (EAS). The non-parametric Mann-Kendall test and the non-parametric Sen's 11 method have been used to study linear trends for pollen season timing and intensity, and 12 13 for temperature and rainfall. Significance was determined using the F-test. Spearman analyses were applied to test for correlations between pollen season parameters and 14 15 weather-related factors before and over the pollen season. The results obtained suggest that flowering has delayed over recent years, especially for trees with a bloom closer to 16 17 spring (poplar and elm). Earlier flowering species are more influenced by the meteorological parameters before the flowering. However, species blooming later are 18 more influenced by the meteorological parameters during the pollen season. 19 Meteorological parameters affect more to the interior cities than the coastal city. 20

KEYWORDS: Winter flowering trees, Pollen season, Climate effects, Meteorological
 factors, Aerobiology

23

MAIN TEXT

24 Introduction.

During the last few years the impact of climate change on ecosystems has prompted growing research into the effect of climate on plant phenology. Numerous studies have focused on analyzing possible trends and modeling the response of plants to the climate (Walter, 2010; Ziello et al., 2012; Bogawski et al., 2014; Heap et al., 2014; Galán et al., 2016).

A number of authors have confirmed a generalized advance in flowering (Gordo & Sanz, 1 2 2010; Anderson et al., 2012; Bellard et al., 2012; Cook et al., 2012; Franks et al., 2014) as well as greater flowering intensity (Ziello et al., 2012). In southern Europe, this 3 generalized advance in flowering has been reported, especially in woody species 4 5 blooming in early spring (García-Mozo et al., 2010a, 2010b; Fernández-Llamazares et al., 2014), some of them with clear trends on flowering intensity (Galán et al., 2016; Recio 6 7 et al., 2018). Although contrary tendencies have been observed in some species of winter flowering (Mercuri et al., 2013) 8

Several studies have highlighted the importance of temperature patterns for the tree's 9 phenological development (Emberlin et al., 2002 and 2007; Mutke et al. 2003; Ariano et 10 al., 2010; García-Mozo et al. 2011). In south Spain, researches have also highlighted the 11 important contribution of rainfall to plant phenology, and various papers have paid special 12 attention to changes in precipitation and water availability as a major driver of climate 13 14 change in the Mediterranean area (Peñuelas et al., 2004; Tormo-Molina et al., 2010; García-Mozo et al., 2010b). Climate change has had a particularly marked effect on 15 16 southern Spain, increasing temperatures and reducing rainfall (IPCC, 2014). Rainfall has 17 also become increasingly torrential in recent years (Priego et al., 2017; Rodríguez-Solà et al., 2017). 18

Airborne pollen monitoring is regarded as an effective tool for studying the reproductive
phenology of anemophilous plant, especially as a bioindicator of their behavior in areas
where there are important variations on flowering (Fernández-Llamazares et al., 2014).

The presence of airborne pollen in the atmosphere is directly affected by weather conditions, not only by daily changes, but also by seasonal climatic trends (Mercuri et al., 2016). On the other hand, some authors also attribute these trends to changes on other components of climate change, i.e. the NAO index or an increase in anthropogenic CO₂
emissions (Rogers et al., 2006; Levetin & Van de Water, 2008; Galán et al., 2016) and
other human activities (Cariñanos & Casares-Porcel, 2011; Oteros et al., 2013; VelascoJiménez et al., 2013; Mercuri et al., 2016).

5 Longer pollen seasons with higher concentrations of airborne pollen, due to variations in climate, may also affect the prevalence and severity of allergic diseases. Indeed, there is 6 evidence that climate change will influence aeroallergens by altering the amounts, the 7 8 allergenicity, and the pollen season, as well as the distribution and other attributes of pollen releasing plants (Ariano et al., 2010). Recent studies highlight the links between 9 global climate change and respiratory allergies, showing that allergic disease is becoming 10 increasingly prevalent, affecting the quality of life of many millions of individuals all 11 over the world (D'Amato et al., 2016; Bousquet et al., 2017). 12

During winter months, main airborne pollen types detected in Andalusian cities are, in order of importance, Cupressaceae, *Populus, Ulmus, Fraxinus* and *Alnus* (Trigo et al., 2008; Martínez-Bracero et al., 2015). Cupresaceae pollen is considered highly allergenic and is the main cause of winter pollinosis in the population (Di Felice et al., 2001; Charpin et al., 2005; Díaz de la Guardia et al., 2006); the other pollen types are not very important from the point of view of allergies, although they have been described by the literature as allergens (Lorenzoni-Chiesura et al., 2000; Cariñanos & Casares-Porcel, 2011)

The main goal of this paper was to investigate variations in pollen seasons of the principal winters flowering trees in Andalusia (Cupressaceae, *Populus, Ulmus, Fraxinus* and *Alnus*) and charting trends over the last twenty four years (1994-2017). Another objective has been to analyze how the meteorological parameters have influenced in the seasonal airborne pollen.

1 Materials and Methods.

2 Study area

Airborne pollen was recorded in three cities in the southern Spanish region of Andalusia:
Cordoba, Granada and Malaga (Figure 1). Some geographical and meteorological
information on these sampling sites is provided in Table 1.

Andalusia has a Mediterranean climate. Malaga is a coastal city, with mild temperatures
reflecting a strong maritime influence. Cordoba is an inland city located in the valley of
the Guadalquivir River, with mild winters and very hot summers. Granada is also an
inland city but it is located next to Sierra Nevada, so the winters are colder than in
Cordoba (Agencia Estatal de Meteorología, 2010).

11 Meteorological parameters

Meteorological parameters during pollen season in winter flowering trees have been considered in the present study, over 1994-2017. In the same way, meteorological parameters of the three months before flowering, over 1993-2016, have been taken into account, since some studies indicate their influence on the pollen season (Recio et al., 2018, Cook et al., 2012; Tormo-Molina et al., 2010; Emberlin et al., 2002 and 2007).

Mean daily temperature (TM=maximum temperature, Tm=minimum temperature,
T=mean temperature) and accumulative daily rainfall (R) data were obtained from the
regional weather station network run by the Spanish Meteorological Agency (AEMet).
Table 1 shows the geographical characteristics of each meteorological station, located in
the respective airports of each city.

22 Airborne pollen

Daily mean pollen concentrations corresponding to five principal arboreal pollen types
 flowering at winter (Trigo et al., 2008; Martínez-Bracero et al., 2015) were utilized in this
 survey: *Alnus*, Cupressaceae, *Fraxinus*, *Populus* and *Ulmus*.

These pollen concentrations were recorded over a 24-year-period (1994–2017) using
Hirst-type volumetric spore traps (Hirst, 1952), following the standardized methodology
of the Spanish Aerobiology Network (REA) (Galán et al. 2007) and in compliance with
the minimum requirements set out by the European Aerobiology Society (EAS) (Galán
et al., 2014).

9 Table 2 shows the species included in ecah pollen type present in the studied cities as well
10 as Mean and Standard Deviation (SD) of annual pollen integral (APIn) in each city. All
11 *Fraxinus* species are considered as anemophilous except *F. ornus* (Dommee et al., 1999),
12 so it can be considered that this specie as low contribution to airborne pollen
13 concentration.

Different methods were tested for the calculation of the pollen season and the one that best suited reality was used in each case. This fact did not affect change in the results because the same method has been used for the same pollen type to calculate the pollen season every year. The pollen season start (PSS) and end (PSE) was taken as the day on which specific daily pollen concentrations (pollen grains/m³) were reached, as a function of intensity and timing flowering:

- Cupressaceae: Start >30; End <30.
- Alnus and Ulmus: Start = first winter day with pollen grains/m³; End = last
 spring day with pollen grains/m³.
- Fraxinus and Populus: Start = 1 pollen grain/m³ + 5 days with 1 or more pollen grains/m³; End = 1 pollen grain/m³ + 5 days with counts below this level.

Cupressaceae pollen type remains in the air over a long period of the year, even out of 1 2 season. For this reason, to avoid very low concentrations out season, sometimes due to 3 re-suspension, we have chosen a minimum concentration to determinate the pollen season. Although in other studies a level of 50 pollen grains has been proposed for this 4 pollen type (Velasco et al., 2013), we have considered a lower level of just 30 (Mesa et 5 al., 2003) to equate conditions in the 3 study cities. For *Fraxinus* and *Populus* pollen types 6 7 the methodology proposed by Velasco-Jiménez et al (2013) has been followed because they have a short pollen season and very well delimited. In these cases, the start date was 8 defined as the first day on which a daily pollen concentration of at least 1 pollen grain/m³ 9 was followed by five days with 1 or more pollen grains/ m^3 and the end of pollen season 10 was defined as the last day on which a daily concentration of at least 1 pollen grain/m³ 11 12 was recorded, followed by five days with concentrations below this level. Finally, Alnus 13 and Ulmus pollen types have low concentrations in the study cities; even, with no pollen days during the pollen season. For this reason it has been necessary to choose a new 14 15 method for the calculation of the pollen season, according to the characteristics of their pollen seasons. The start of pollen season have been considered the first winter day when 16 pollen was detect and the end of pollen season the last spring day with pollen. 17

The Main Pollen Season (MPS) refers to the number of days for pollen season. The Annual Pollen Integral (APIn) was defined as the annual sum of daily values (measured in pollen grains/m³). The Peak Day (PD) refers to the day of the year in which the Maximum Daily pollen Concentration (MDC) was reached.

22 Statistical analysis

The non-parametric Mann-Kendall test for testing the presence of the monotonicincreasing or decreasing trends and the non-parametric Sen's method for estimating the

slope of a linear trend have been used to study annual linear trends for main pollen season
 timing and intensity, and also for temperature and rainfall. Significance was determined
 using the F-test.

On the other hand, Spearman analyses were applied to test for possible correlations
between pollen parameters and climatic-related factors before and over the main pollen
season.

7 **Results.**

8 *Meteorological parameters trends during the study years.*

9 The temperature during the autumn (three months before pollen winter season) is 10 increasing during the last 23 years with significant values in Granada for minimum 11 (+0.094°C/year) and average temperature (+0.064°C/year). During the main pollen 12 season, from December to March, it has been observed decreasing trend temperatures, 13 reaching significant values in the cities of Cordoba and Granada for maximum 14 temperature (-0.052°C/year in Córdoba and -0.091°C/year in Granada) and average 15 temperature (-0.083°C/year in Córdoba and -0.065°C in Granada) (Table 3).

16 *Pollen seasons during the study years.*

According to Figure 2, during the study period (1994-2017), main pollen seasons generally extended from December to March in the studied cities. The first flowering tree was *Fraxinus*, whose pollen grains were detected since the beginning of December; it is followed by *Alnus*, detecting pollen in late December; Cupressaceae at the beginning of the year; and *Ulmus* and *Populus* pollen was not detected until February. In general, the more abundant pollen types in all cities have been Cupressaceae and *Populus*. The other pollen types have presented low concentrations, with a daily average concentration of less than 15 pollen grains/m³ of air, even not exceeding 3 grains of pollen daily/m³ of air in
the case of *Alnus*.

3 *Trends in main pollen season during the study years.*

4 Slope, R^2 and significance (p) values for the temporality and intensity of the pollination (PSS, PSE, APIn, PD, MCD) in each city are summarized in Table 4. Pollen season start 5 tends towards to be delayed for Alnus in Cordoba and Malaga, Populus in Malaga and 6 7 Ulmus in Granada; while it tends to be advanced for Cupressaceae in Granada and Fraxinus in Córdoba. Pollen season end tends to be delayed for Alnus, Cupressaceae and 8 9 Ulmus in Granada and Populus in Cordoba and Granada; while it tends towards to be advance for Alnus in Malaga. Annual pollen integral are increasing for Alnus, 10 Cupressaceae and Fraxinus in Granada and Populus and Ulmus in Cordoba; while it is 11 12 decreasing for Cupressaceae in Malaga and Ulmus in Granada. Maximum Daily pollen Concentration is increasing for *Alnus* in Granada, Cupressaceae and *Ulmus* in Cordoba 13 and Granada and Populus in Cordoba; while it is decreasing for Cupressaceae in Malaga. 14 Neither result was statistically significant for date of Peak day. 15

16 *Correlation between the starting of pollen season (PSS) or annual pollen integral (APIn)*

17 *and Climatic parameters.*

Results of Spearman correlation analyses between PSS and APIn of pollen types studied and the climatic parameters (TM, Tm, T and R) before and during main pollen season in each city over the study period are shown in Table 5. As general results, and in spite of not obtaining significant values in the majority of the cases, it has been observed that these climatic parameters affect more to the interior cities (Cordoba and Granada) than the coastal city (Malaga). It is observed that higher rainfall and temperatures prior to flowering cause a delay in the PSS and an increase in the APIn. Similarly, higher temperatures during the pollen season, greater delay in the PSS and an increase in the
 APIn.

3 Discussion.

This study focused on the most frequent winter pollen types in the atmosphere of south 4 Spain. Different pollen season characteristics (PSS, PSE, APIn, and MCD) were analyzed 5 as indicators of timing flowering and intensity in these anemophilous plants in areas 6 surrounding the sampling station. In this sense, foreground the higher pollen 7 8 concentration of Cupressaceae was detected in Granada, since several cypress species are 9 widely used as ornamental in this city (Díaz de la Guardia et al., 2006; Velasco-Jiménez 10 et al., 2014). In the same way, pollen concentrations of Alnus, Populus and Ulmus pollen types, typical riverside species, are similar in Cordoba and Granada, both cities crossed 11 12 by rivers. Nevertheless, pollen concentrations of these species are lower in Malaga because they are worse represented in this city (Velasco-Jiménez et al., 2014). In addition, 13 it is a coastal city and suffers from the influence of wind direction, which usually has a 14 southeast component (from the sea) and therefore the pollen concentration detected is 15 much lower. 16

The present study examined data over the same 24-year period, and generally speaking, 17 PSS and PSE have delayed over recent years, although the delay is more evident for trees 18 with a bloom closer to spring (Populus sp. and Ulmus sp.). This result is different from 19 other studies in central-Europe (Clot et al., 2003; Bogawski et al., 2014), with a 20 21 predominantly Euro-Siberian temperate climate. In other studies carried out in southern Spain (García-Mozo et al., 2010a, 2010b; Tormo-Molina et al., 2010), it is about other 22 species blooming later in spring (*Quercus* sp. and *Platanus* \times *hispanica Mill. ex* 23 Münchh.,), even, sometimes with no significant results for Cupressaceae pollen type. 24

However, we have observed that over last years there is a trend to advance the beginning
 of pollination of some anemophylous taxa with winter flowering in Granada
 (Cupressaceae) and Cordoba (*Fraxinus*) because temperature during previous months
 (autumn) tends to increase, especially in Granada.

The APIn, the annual sum of daily airborne pollen concentration, can provide information
on the distribution and flowering intensity of wind-pollinated plants (Galán et al., 2016).
For this parameter, different results have been obtained, depending on the city in question,
and no clear trends, probably due to the management on these ornamental trees, with
periodic pruning, which alter the formation of flowers and, therefore, pollen production
(Cariñanos et al., 2011).

We also think that the increase in temperature in months before flowering (whose trend 11 12 has been significant and positive in Granada) favors the formation and differentiation of sexual reproductive buds, which implies a higher number of flowers and therefore more 13 pollen. This fact, together with a slight decrease in the rainfall during the pollen season 14 could favor this increase of the APIn. This reality has been observed in the case of 15 Populus and Ulmus pollen types in Córdoba and in Fraxinus, Alnus and Cupressaceae 16 17 pollen types in Granada which, as previously mentioned, this last is widely used as an ornamental species. 18

However, a decrease was observed in the case of *Ulmus* pollen type in Granada, probably due to the effects of dutch elm disease (González-Ruiz et al., 1998); but not really in Córdoba, probably because the damage of this pest has been less here or because the trees are already recovering. The APIn for Cupressaceae pollen type has decreased in the city of Malaga, a result that coincides with that obtained by Galán et al. (2016) for this city.

About the influence of meteorological conditions during this period of years, it has been 1 2 observed that they affect more to the interior cities (Cordoba and Granada) than the 3 coastal city (Malaga). This could be due to the fact that the sea exerts a mitigating effect on the climate, so that the meteorological changes have not been appreciated too much in 4 5 this city. It has been also observed that a higher temperature before flowering causes a delay in the start of the pollen season. This result coincides with other studies carried out 6 7 with winter flowering species in Italy, where this delay in the pollen season was already observed due to higher temperatures in autumn (Mercuri et al., 2013 and 2016). An 8 9 explanation of this could be that these trees need a cold blow to break the dormancy of 10 their buds (Perry, 1971) and if the temperatures are not low enough this break of 11 dormancy is delayed. On the other hand, a higher precipitation prior to flowering is also causing a delay in the PSS. This could be due to the fact that on rainy days the 12 13 temperatures in winter are less cold and this would be delaying the dormancy, as previously were mentioned. 14

On the other hand, in the interior cities, an effect of the altitude has also been observed,so in the city of Granada a greater effect of the climate was appreciated.

Finally, it has been observed that these trends could not to be solely due to climate, or to any specific component of climate change, and they could be dictated more by local changes due to human activity. For example, the adoption of gardening practices such as pruning before flowering can reduced flowering intensity, leading to lower airborne pollen concentrations than in other cities. In the same way, an increase in the number of individuals planted in parks and gardens step up the pollen concentration in the air (Cariñanos et al., 2011; Velasco-Jiménez et al., 2014).

24 Conclusions.

Pollen concentration of each city is a reflection of the ornamental and natural flora present
 in each one of them.

Generally, more clear trends have been observed in inland cities than in coastal one, 3 where the sea exerts a mitigating effect on the climate. An effect of the altitude has also 4 5 been observed, so at higher altitude better trends have been observed. A delay on flowering during the study period, especially for trees with a bloom closer to 6 7 spring (poplar and elm) has been observed. An increase of the temperature and precipitation in the months before flowering has been 8 able to cause the increase of the pollen concentrations in many of the studied trees. 9 10 However, this could also be the cause of the delay in the pollen season due to the fact that 11 these trees need a cold blow to break the latency. 12 The trends observed could be also dictated by local changes due to human activity.

13 Bibliography.

14 Agencia Estatal de Meteorología. (2010). *Guía Resumida del Clima en España* (1981–
15 2010).

Anderson, J. T., Inouye, D. W., McKinney, A. M., Colautti, R. I., & Mitchell-Olds, T.
(2012). Phenotypic plasticity and adaptive evolution contribute to advancing flowering
phenology in response to climate change. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20121051.

Ariano R, Canonica GW, Passalacqua G. 2010. Possible role of climate changes in
variations in pollen seasons and allergic sensitizations during 27 years. *Annals of Allergy, Asthma & Immunology*, 104(3), 215-222.

1	Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts
2	of climate change on the future of biodiversity. <i>Ecology letters</i> , 15(4), 365-377.
3	Bogawski, P., Grewling, Ł., Nowak, M., Smith, M., & Jackowiak, B. (2014). Trends in
4	atmospheric concentrations of weed pollen in the context of recent climate warming in
5	Poznań (Western Poland). International journal of biometeorology, 58(8), 1759-1768.
6	Bousquet, J., O'Hehir, R. E., Anto, J. M., D'Amato, G., Mösges, R., Hellings, P. W., &
7	Sheikh, A. (2017). Assessment of thunderstorm-induced asthma using Google Trends.
8	Journal of Allergy and Clinical Immunology, 140(3), 891-893.
9	Cariñanos, P., & Casares-Porcel, M. (2011). Urban green zones and related pollen allergy:
10	a review. Some guidelines for designing spaces with low allergy impact. Landscape and
11	Urban Planning, 101(3), 205-214.
12	Cariñanos, P., & Casares-Porcel, M. (2011). Urban green zones and related pollen allergy:
13	A review. Some guidelines for designing spaces with low allergy impact. Landscape and
14	Urban Planning, 101(3), 205-214.
15	Charpin, D., Calleja, M., Lahoz, C., Pichot, C., & Waisel, Y. (2005). Allergy to cypress
16	pollen. Allergy, 60(3), 293-301.
17	Clot, B. (2003). Trends in airborne pollen: an overview of 21 years of data in Neuchâtel
18	(Switzerland). Aerobiologia, 19(3), 227-234.
19	Cook, B. I., Wolkovich, E. M., & Parmesan, C. (2012). Divergent responses to spring and
20	winter warming drive community level flowering trends. Proceedings of the National
21	Academy of Sciences, 109(23), 9000-9005.

1	D'Amato, M., Vitale, C., Molino, A., Sanduzzi, A., Mormile, M., Vatrella, A., &
2	D'Amato, G. (2016). Climate change, thunderstorms and asthma attacks during the pollen
3	seasons. International Journal on Immunorehabilitation, 18(2), 97-100.
4	Di Felice, G., Barletta, B., Tinghino, R., & Pini, C. (2001). Cupressaceae pollinosis:
5	identification, purification and cloning of relevant allergens. International archives of
6	allergy and immunology, 125(4), 280-289.
7	Díaz de la Guardia C, Alba F, de Linares C, Nieto-Lugilde D, López Caballero J. (2006).
8	Aerobiological and allergenic analysis of Cupressaceae pollen in Granada (Southern
9	Spain). J Investig Allergol Clin Immunol 16(1), 24-33.
10	Díaz de la Guardia, C., Alba-Sánchez, F., Linares Fernández, C. D., Nieto-Lugilde, D.,
11	& López Caballero, J. (2006). Aerobiological and allergenic analysis of Cupressaceae
12	pollen in Granada (Southern Spain). J Investig Allergol Clin Immunol, 16(1): 24-33
13	Dommee, B., Geslot, A., Thompson, J. D., Reille, M., & Denelle, N. (1999). Androdioecy
14	in the entomophilous tree Fraxinus ornus (Oleaceae). The New Phytologist, 143(2), 419-
15	426.
16	Emberlin J, Detandt M, Gehrig R, Jaeger S, Nolard N, Rantio-Lehtimäki A. (2002).
17	Responses in the start of Betula (birch) pollen seasons to recent changes in spring
18	temperatures across Europe. Int J Biometeorol 46,159–170.
19	Emberlin, J., Smith, M., Close, R., & Adams-Groom, B. (2007). Changes in the pollen
20	seasons of the early flowering trees Alnus spp. and Corylus spp. in Worcester, United
21	Kingdom, 1996–2005. International journal of biometeorology, 51(3), 181.

1	Fernánc	lez-Llama	zares,	A., Beli	monte, J., E	Boada, M., & F	raixe	edas, S	5. (2014). Airbo	rne
2	pollen	records	and	their	potential	applications	to	the	conservation	of
3	biodive	rsity. Aero	biolog	ia, 30(2), 111-122.					

- 4 Franks, S. J., Weber, J. J., & Aitken, S. N. (2014). Evolutionary and plastic responses to
- 5 climate change in terrestrial plant populations. *Evolutionary Applications*, 7(1), 123-139.
- Galán C, Cariñanos P, Alcázar, Domínguez-Vilches E. D. (2007). Spanish Aerobiology *Network (REA): management and quality manual.* Servicio de Publicaciones,
 Universidad de Córdoba.
- 9 Galán C, Smith M, Thibaudon M, Frenguelli G, Oteros J, Gehrig R & EAS QC Working
 10 Group. (2014). Pollen monitoring: minimum requirements and reproducibility of
 11 analysis. *Aerobiologia*, 30(4), 385-395.
- Galán, C., Alcázar, P., Oteros, J., García-Mozo, H., Aira, M. J., Belmonte, J., ... & PérezBadía, R. (2016). Airborne pollen trends in the Iberian Peninsula. *Science of the Total Environment*, 550, 53-59.
- García-Mozo H, Mestre A, Galán C. 2011. Climate change in Spain: phenological trends
 in southern areas. *Climate Change–Socioeconomic effects*, 237.
- García-Mozo, H., Galán, C., Alcázar, P., de la Guardia, C. D., Nieto-Lugilde, D., Recio,
 M., ... & Domínguez-Vilches, E. (2010a). Trends in grass pollen season in southern Spain. *Aerobiologia*, 26(2), 157-169.
- García-Mozo, H., Mestre, A., & Galán, C. (2010b). Phenological trends in southern
 Spain: a response to climate change. *Agricultural and Forest Meteorology*, 150(4), 575580.

1	González-Ruiz, R. (1998). Consideraciones sobre la situación actual y evolución de la
2	grafiosis del olmo, Ophíostoma novo-u/mí, en la Alhambra y el Generalife (Granada,
3	1997). <i>Ecología</i> (12), 307-318.
4	Gordo, O., & Sanz, J. J. (2010). Impact of climate change on plant phenology in
5	Mediterranean ecosystems. Global Change Biology, 16(3), 1082-1106.
6	Heap, M. J., Culham, A., Lenoir, J., & Gavilán, R. G. (2014). Can the Iberian floristic
7	diversity withstand near-future climate change? Open Journal of Ecology, 4(17), 1089-
8	1101.
9	IPCC, 2014. Climate change 2014: impacts, adaptation, and vulnerability. Fifth
10	assessment report (http://www.ipcc.ch/report/ar5/syr/acesed on February 2015).
11	Levetin, E., & Van de Water, P. (2008). Changing pollen
12	types/concentrations/distribution in the United States: fact or fiction? Current allergy and
13	asthma reports, 8(5), 418-424.
14	Lorenzoni-Chiesura, F., Giorato, M., & Marcer, G. (2000). Allergy to pollen of urban
15	cultivated plants. Aerobiologia, 16(2), 313-316.
16	Martínez-Bracero M., Alcázar P., de la Guardia C. González-Minero F.J., Ruiz L., Pérez
17	M. T., Galán C. (2015). Pollen calendars: a guide to common airborne pollen in
18	Andalusia. Aerobiologia, 31(4), 549-557.
19	Mercuri, A. M., Torri, P., Casini, E., & Olmi, L. (2013). Climate warming and the decline
20	of Taxus airborne pollen in urban pollen rain (Emilia Romagna, northern Italy). Plant
21	<i>Biology</i> , 15, 70-82.

1	Mercuri, A., Torri, P., Fornaciari, R., & Florenzano, A. (2016). Plant Responses to
2	Climate Change: The Case Study of Betulaceae and Poaceae Pollen Seasons (Northern
3	Italy, Vignola, Emilia-Romagna). Plants, 5(4), 42.

Mesa, J. A. S., Smith, M., Emberlin, J., Allitt, U., Caulton, E., & Galan, C. (2003).
Characteristics of grass pollen seasons in areas of southern Spain and the United
Kingdom. *Aerobiologia*, 19(3-4), 243-250.

Mutke S, Gordo J, Climent J, Gil L. 2003. Shoot growth and phenology modelling of
grafted Stone pine (*Pinus pinea* L.) in Inner Spain. *Annals of Forest Science*, 60(6), 527537.

- Oteros, J., García-Mozo, H., Hervás, C., & Galán, C. (2013). Biometeorological and
 autoregressive indices for predicting olive pollen intensity. *International journal of biometeorology*, 57(2), 307-316.
- 13 Peñuelas, J., Filella, I., Zhang, X., Llorens, L., Ogaya, R., Lloret, F., ... & Terradas, J.
- 14 (2004). Complex spatiotemporal phenological shifts as a response to rainfall changes.
- 15 *New Phytologist*, 161(3), 837-846.
- 16 Perry, T. O. (1971). Dormancy of trees in winter. *Science*, 171(3966), 29-36.
- Priego, E., Jones, J., Porres, M. J., & Seco, A. (2017). Monitoring water vapour with
 GNSS during a heavy rainfall event in the Spanish Mediterranean area. *Geomatics*, *Natural Hazards and Risk*, 8(2), 282-294.
- Recio M, Picornell A, Trigo MM, Gharbi D, García-Sánchez J, Cabezudo B. (2018).
 Intensity and temporality of airborne *Quercus* pollen in the southwest Mediterranean
 area: Correlation with meteorological and phenoclimatic variables, trends and possible
 adaptation to climate change. *Agricultural and Forest Meteorology* 250–251, 308-318

1	Rodríguez-Solà, R., Casas-Castillo, M. C., Navarro, X., & Redaño, A. (2017). A study of
2	the scaling properties of rainfall in Spain and its appropriateness to generate intensity-
3	duration-frequency curves from daily records. International Journal of Climatology,
4	37(2), 770-780.

5	Rogers, C. A., Wayne, P. M., Macklin, E. A., Muilenberg, M. L., Wagner, C. J., Epstein,
6	P. R., & Bazzaz, F. A. (2006). Interaction of the onset of spring and elevated atmospheric
7	CO2 on ragweed (Ambrosia artemisiifolia L.) pollen production. Environmental health
8	perspectives, 114(6), 865.

9 Tormo-Molina, R., Gonzalo-Garijo, M. A., Silva-Palacios, I., & Muñoz-Rodríguez, A. F.

(2010). 5 General Trends in Airborne Pollen Production and Pollination Periods at a
Mediterranean Site (Badajoz, Southwest Spain). *Journal of investigational allergology &*

- 12 *clinical immunology*, 20(7), 567.
- Trigo, M. M., Jato, V., Fernández, D., Galán, C. (2008). *Atlas aeropalinológico de España*. Espana: Universidad de Leon, 111.
- Velasco-Jiménez M.J., Alcázar P., Valenzuela L.R., Gharbi D., Díaz de la Guardia C.,
 Galán C. (2018). Pinus pollen season trend in South Spain. *Plant Biosystems*, 152: 657665.
- Velasco-Jiménez, M. J., Alcázar, P., Domínguez-Vilches, E., & Galán, C. (2013).
 Comparative study of airborne pollen counts located in different areas of the city of
 Córdoba (south-western Spain). *Aerobiologia*, 29(1), 113-120.
- 21 Velasco-Jiménez, M. J., Alcázar, P., Valle, A., Trigo, M. M., Minero, F., Domínguez-
- 22 Vilches, E., & Galán, C. (2014). Aerobiological and ecological study of the potentially
- allergenic ornamental plants in south Spain. Aerobiologia, 30(1), 91-101.

- Walther, G. R. (2010). Community and ecosystem responses to recent climate change.
 Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1549), 2019 2024.
- 4 Ziello, C., Sparks, T. H., Estrella, N., Belmonte, J., Bergmann, K. C., Bucher, E., ... &
- 5 Gehrig, R. (2012). Changes to airborne pollen counts across Europe. *PloS one*, 7(4),

6 e3407.

Tables:

Table 1. Geographical and meteorological characteristics of the study cities. T (Mean annual average temperature), TM (Mean annual maximum temperature), Tm (Mean annual minimum temperature), R (Total annual Rainfall). Meteorological data from 1981-2010. AEMET (State weather agency). Geographical characteristics of the meteorological stations.

			City		Meteorological stations		
	Coordinate	Altitude (m)	T (°C)	TM (°C)	Tm (°C)	R (mm)	Coordinate Altitude (m)
Cordoba	37°50'N, 4°45'W	122.2	18.2	25.1	11.4	605	37° 50' 56" N, 4° 50' 48" W 90
Granada	37°11'N, 3°35'W	689.1	15.4	23	7.8	365	37° 11' 23" N, 3° 47' 22" W 567
Malaga	36°47'N, 4°19'W	9.7	18.5	23.3	13.7	534	36° 39' 58" N, 4° 28' 56" W 5

Table 2. Species of the selected pollen types present in the study cities. Mean and Standard Deviation (SD) of pollen in each city during the study period.

Dollon tunos			ç	Species			
Pollen types	Co	ordoba	(Granada		Malaga	
Pollen types - Alnus - Cupressaceae - Fraxinus - Populus - Ulmus -	A. glutinosa (L.) Ga	aertner	A. glutinosa (L.) C	Baertner	A. glutinosa (L.)	Gaertner	
	Mean: 66	SD: 31.822	Mean: 21	SD: 17.575	Mean: 35	SD: 20.797	
	Cupressus semper Hesperocyparis ari	virens L. zonica (Greene) Bartel	Cupressus lusitani C. sempervirens L	ica Miller	Cupressus lusitar C. sempervirens 1	<i>nica</i> Miller L.	
Cupressaceae	H. glabra (Sudw.) I H. macrocarpa (Ha	Bartel. artw. ex Gordon) Bartel	Hesperocyparis. a H. macrocarpa (H	rizonica (Greene) Bartel Iartw. ex Gordon) Bartel	Hesperocyparis arizonica (Greene) Barter H. macrocarpa (Hartw. ex Gordon) Barte		
	Mean: 7070	SD: 3805.2	Mean: 17975	SD: 8318	Mean: 5411	SD: 3374.9	
Fraxinus	F. angustifolia Vah F. excelsior L. F. ornus L	1	F. angustifolia Va F. ornus L.	hl	F. angustifolia Vahl		
	Mean: 298	SD: 233.29	Mean: 121	SD: 63.863	Mean: 85	SD: 34.468	
	<i>P. x canadensis</i> M <i>P. alba</i> bolleana (L	oench auche) & Otto	<i>P. alba</i> bolleana (l <i>P. alba</i> L.	Lauche) & Otto			
Populus	P. alba L. P. caroliniana Hort P. nigra L. P. simonii Carrieré P. x canascans (Ait	t. ex McMinn & Maino	P. caroliniana Ho P. nigra L. P. simonii Carriero P. tremula L.	rt. ex McMinn & Maino é	<i>P. x canadensis</i> Moench <i>P. alba</i> L.		
	Mean: 1075	SD: 662.5	Mean: 1148	SD: 580.94	Mean: 122	SD: 45.504	
Ulmus	U. americana L. U. glabra Hudson U. minor Miller U. pumila L.		<i>U. glabra</i> Hudson <i>U. minor</i> Miller		U. minor Miller		
	Mean: 220	SD: 154.98	Mean: 262	SD: 179.16	Mean: 23	SD: 14.67	

Table 3. Slope values, R^2 and significance (p) for the meteorological parameters (Maximum temperature – TM, Minimum temperature – Tm, Average temperature – T, Rain – R) during main pollen season (December-March) and before (September-November) in each city. **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level; * Correlation is significant at the 0.1 level

			Befor	e MPS		During MPS						
		TM	Tm	Т	R	ТМ	Tm	Т	R			
	Slope	0.040	0.017	0.003	-0.393	-0.052	-0.056	-0.083	-1.867			
Cordoba	\mathbb{R}^2	0.062	0.015	0.001	0.001	0.157	0.070	0.348	0.005			
	р	0.242	0.568	0.916	0.886	0.055+	0.213	0.002**	0.746			
	Slope	0.066	0.094	0.064	1.008	-0.091	-0.005	-0.065	-0.179			
Granada	\mathbf{R}^2	0.117	0.207	0.126	0.012	0.223	0.001	0.175	0.000			
	р	0.102	0.026*	0.089+	0.608	0.020*	0.904	0.042*	0.966			
	~ 4					· · · · -						
	Slope	0.047	-0.023	0.000	0.971	-0.007	-0.038	-0.028	-0.733			
Malaga	\mathbb{R}^2	0.069	0.017	0.000	0.003	0.006	0.070	0.074	0.001			
	р	0.214	0.544	0.996	0.811	0.728	0.212	0.199	0.862			

Table 4. Slope , R^2 and significance (p) values for the seasonality and intensity of the pollination (Pollen Season Start –PSS-, Pollen Season End – PSE-, Annual Pollen Integral –APIn-, Maximum Daily pollen Concentration -MCD) of pollen types studies in each city. ***Correlation is significant at the 0,001 level; **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.1 level

	Cordoba						Gran	Malaga					
		PSS	PSE	APIn	MCD	PSS	PSE	APIn	MCD	PSS	PSE	APIn	MCD
S	Slope	-1,345						3,466					
uuixe.	R2	0,222						0,147					
F_{P}	р	0,020*						0,064+					
	Slope	0,897					2,971	2,334	0,440	1,859	-1,248		
41nus	\mathbb{R}^2	0,210					0,160	0,215	0,215	0,276	0,165		
~	р	0,024*					0,053+	0,022*	0,023*	0,008**	0,049*		
seae	Slope				80,409	-8,527	3,690	1445,442	103,451			-391,670	-33,442
essac	\mathbb{R}^2				0,229	0,491	0,817	0,579	0,381			0,163	0,205
Cupr	р				0,018*	0,000***	0,000***	0,000***	0,001**			$0,050^{+}$	0,026*
S	Slope		0,870	56,413	11,527		0,981			0,443			
nInde	\mathbb{R}^2		0,266	0,363	0,295		0,391			0,121			
P_{c}	р		0,001**	0,002**	0,006**		0,001**			0,095+			
s	Slope			9,705	1,291	0,749	0,588	-10,953	-1,461				
Ulmu	\mathbb{R}^2			0,196	0,209	0,294	0,166	0,187	0,194				
	р			0,030*	0,023*	0,006**	0,048*	0,035*	0,031*				

Table 5. Spearman correlations analysis between Pollen Season characteristics (Pollen Season Start –PSS-, Annual Pollen Integral –APIn), of pollen types studies and the meteorological parameters (Maximum temperature – TM, Minimum temperature – Tm, Average temperature – T, Rain - R) before and during main pollen season. ***Correlation is significant at the 0,001 level; **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.1 level.

		Before MPS							During MPS					
		Cordoba		Granada		Malaga		Cordoba		Granada		Malaga		
		PSS	APIn	PSS	APIn	PSS	APIn	PSS	APIn	PSS	APIn	PSS	APIn	
Fraxinus	TM							$(+)^{+}$						
	Tm					(+)*								
	Т							(+)*	$(+)^{**}$					
	R													
Alnus	TM													
	Tm	(+)*		$(+)^{***}$		(+)*			(+)*					
	Т			(+)*		(+)*			$(+)^{***}$					
	R	(+)*		(+)*	. <u>.</u>									
Cupressaceae	ΤM													
	Tm													
	Т													
	R			(+)+										
Populus	ΤM				$(+)^{+}$		$(+)^{+}$			(+)**				
	Tm			(+)*				(+)**						
	Т	(+)**	$(+)^+$					(+)*		(+)**				
	R	(+)*												
Ulmus	TM													
	Tm		$(+)^{**}$					(+)*						
	Т		$(+)^{**}$					(+)*						
	R	(+)*	$(+)^{+}$											

Figures:

Figure 1. Location of the study cities in south Spain.





Figure 2. Average Daily Pollen Concentration of each pollen types in each city during the study years.