

Environmental analysis of airborne pollen occurrence, pollen source distribution and phenology of *Fraxinus angustifolia*

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Abstract Narrow-leaved ash (*Fraxinus angustifolia*) is a common polygamous tree growing on the banks of rivers in the western Mediterranean region. Pollination occurs during winter, and the tree's pollen is among the most abundant during that season. This work aims to relate the phenology of pollen shedding, source tree distribution, meteorology and airborne pollen occurrence for the species. Aerobiological sampling was conducted in Badajoz (south-western Spain) using a Hirst volumetric sampler over 24 years (1993–2016). Trees were geo-localized in a circle 500 m in diameter surrounding the pollen sampler. During the last two periods, pollination phenology was studied in 10 specimens, five in the surroundings of the pollen station and five 3 km apart, at a frequency of 3–4 days

on average. Moreover, a detailed analysis of pollen occurrence was conducted for these two periods. Daily data for the whole period and hourly data over the last 2 years were used, including pollen monitoring and meteorology. A comparison was made between pollen occurrence and source distribution. The main pollen season lasted on average 53 (28–75) days. Average values were less than 10 grains m⁻³, except for two periods of 23–24 grains m⁻³. Daily data and hourly data correlation with meteorology showed different signs in correlation analysis. Hourly analysis showed that the maximum concentration occurred just after noon. Most pollen was recorded at an average temperature of 9 °C. Analysis of pollen sources and pollen occurrence showed a close relationship between predominant wind directions and tree distribution. Peaks of phenology were not coincident with pollen peaks. No trends in pollination were found. Non-homogeneous distribution of pollen sources for *Fraxinus angustifolia* provided a suitable tool to demonstrate that wind direction plays a relevant role when aerobiological data are interpreted according to source distribution. A limitation in phenology analysis and aerobiological data was noted in the narrow-leaved ash species.

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1 Introduction

The genus *Fraxinus* includes mostly deciduous trees commonly named ashes. More than 40 species have been described, and they are distributed mainly in the Northern Hemisphere, mostly in North America and Eastern Asia (FRAXIGEN 2005). These trees are common in riverside forests in dryer regions and are elements of forests in humid zones, but they are also frequently planted as ornamentals in green urban areas (Wallander 2008). Flowers possess only two stamens emerging in most cases before the leaves unfold. There are a variety of pollination and breeding systems in *Fraxinus*. Approximately one-third of the species are entomophilous, and two-thirds are anemophilous. Most of the anemophilous species are dioecious or polygamous, and many species are morphologically androdioecious. This variety in breeding and pollination systems is a response to their biogeographic history during the Oligocene (Hinsinger et al. 2013).

In Western Europe, three species of *Fraxinus* occur naturally, and some are cultivated as ornamentals. On the Iberian Peninsula, *F. excelsior* grows north into the Eurosiberian Region and *F. angustifolia* occurs in the remainder of the area into the Mediterranean Region; both are polygamous and wind-pollinated trees. A third species, *F. ornus*, androdioic and insect-pollinated, appears mainly in east of Iberian Peninsula (Andrés 2012). For *F. angustifolia*, pollen production per tree was estimated to be approximately 100×10^9 , although it is among the species of Mediterranean trees with more variability (Tormo-Molina et al. 1996b). The relevance in vegetation for the European ash (*Fraxinus excelsior*) is highlighted by its relation to the dieback that threatens these forests (Pautasso et al. 2013), and the success of its reproductive biology is marked mainly by phenology (Bochenek 2011).

Although *Fraxinus* pollen sensitization is detected in patients with respiratory allergies (positive skin tests and/or specific IgE), the clinical relevance of this sensitization is not well established, and the interpretation of the results varies depending on the geographical area and exposure to other pollens of recognized allergenic importance (Hemmer et al. 2000; Niederberger et al. 2002; Poncet et al. 2010; Weber 2014). Data on the prevalence of sensitivity to ash are scarce. In fact, it is not clear whether ash pollen is a primary cause of sensitization or whether it is implied through cross-sensitization of other *Oleaceae*

pollens (as in the Mediterranean–temperate areas) or botanically unrelated plants, such as grasses and birch, due to the presence of pollen panallergens, such as profilins and polcalcins (D’Amato et al. 1998; Niederberger et al. 2002; Palomares et al. 2006; Poncet et al. 2010; Mas et al. 2014a). In a study conducted in 12 cities in Spain in 2003, no significant association was found between *Fraxinus* pollen concentrations and the prevalence of sensitization to *F. excelsior* (ranging from 8 to 56%) in patients with pollinosis (rhinoconjunctivitis and/or asthma) (Moral et al. 2016). Specific allergens have been described in species of *Fraxinus* with evident cross-reactivity with other pollens of the *Oleaceae* family (Barderas et al. 2005, 2006; Rodríguez-Rajo et al. 2010; Mas et al. 2014a, b; Robledo-Retama et al. Robledo-Retana et al. 2015; Torres et al. 2015).

Although for some authors, *Fraxinus* pollen rarely induces allergic respiratory symptoms in Europe (D’Amato et al. 2007), for other authors, the potential role of ash pollinosis may have been underestimated in some zones, such as the Middle European temperate zones, because of an overlapping pollination period with other trees such as birch and other species of the Fagales order. Hemmer et al. (2000) reported in eastern Austria that ash pollen should be considered a relevant allergen in spring pollinosis as, in all, only 20% of positive skin tests to ash appear to result from the cross-sensitization of pollen panallergens (Vara et al. 2016a). Moreover, an ash pollen allergy has been documented in areas without olive in central Europe such as Switzerland (Schmid-Grendelmeier 2012) and Alsace (France), where 18–34% of pollinosis could be attributed to ash pollen (Metz-Favre et al. 2010), as well as in Canada (Dales et al. 2008). Indirectly, the clinical relevance of allergen sensitization has been related to its presence in the atmosphere and drug use to treat symptoms related to this exposure. Thus, in a study in Brussels (Guilbert et al. 2016) and in others in central France (Fuhrman et al. 2007; Caillaud et al. 2015), the presence of *Fraxinus* pollen was associated with an increase in drug consumption to treat respiratory allergy symptoms. In contrast, in Spain, sensitization to *Fraxinus* pollen does not seem clinically relevant in areas of high exposure and sensitization to *Olea* pollen, such as Córdoba (Guerra et al. 1995), or in areas of moderate exposure, such as Navarra (García et al. 2011) or Galicia (Vara et al., 2016b).

One of the first aerobiological studies of *Fraxinus* airborne pollen was developed in Spain using a *Cour*

sampler (Candau et al. 1994). This study showed that dormancy of *F. angustifolia* occurs during the first week with the lowest mean temperature. Furthermore, one and a half or two months later, pollination occurs. Using volumetric Hirst (1952) samplers, Jato et al. (2004) analysed *Fraxinus* airborne pollen from Galicia (north-western Spain) for 5 years to determine the cold and heat required to trigger flowering.

Fraxinus pollen did not show a clear intradiurnal pattern. Pollen occurred in the air during the second half of the day with maximums in the early afternoon in Germany (Simoleit et al. 2016). Kasprzyk et al. (2004) analysed the pollen season of three pollen types, including *Fraxinus*, from seven places in Poland, concluding that regional differences in the start of pollen season in Poland did not exist. Moreover, Puc (2012) concluded after 7 years of study in Szczecin that 50% of the total daily pollen was noted at 14:00; furthermore, mean temperature, total radiation, air pressure, relative humidity and wind speed were the main factors influencing the intradiurnal pollen concentrations in the atmosphere.

Peeters (2000) analysed the meteorological conditions leading to the release of ash pollen in Zurich (Switzerland), concluding that a temperature below -2°C triggers the start of pollination.

Two-hour variations of *Fraxinus* pollen were analysed, and observations of the diurnal peak in the afternoon or even midnight in Bratislava (Slovakia) were noted (Ščevková et al. 2015).

Fraxinus pollen was the third airborne pollen in terms of relevance (Bicakci et al. 2017), showing that the start of pollination occurs at the end of the year in Turkey.

In Southern Hemisphere countries such as Argentina, *Fraxinus* pollen has been detected mainly over one month between September and October with a maximum concentration of more than 2000 grains/m³ and hourly pollen showing a maximum concentration between 10:00 and 14:00 (Nitiu and Mallo 2002; Nitiu et al. 2003). *Fraxinus americana* is a species present in Buenos Aires and is responsible for the high airborne pollen values there (Majas et al. 1992).

In México, Rocha-Estrada et al. (2008) and Calderón-Ezquerro et al. (2016) concluded that *Fraxinus*, represented by *F. uhdei*, had the largest amount of pollen with 28% of the total grains and that it was present in the area in sufficient quantities to be the

cause of allergic disorders in the human population, mainly during February.

In relation to trends in pollination, and according to a study in Europe of 23 pollen types with data over 10–20 years (Ziello et al. 2012), including that of *Fraxinus*, there seemed to be an increasing trend in the yearly amount of airborne pollen for many taxa in Europe, which was more pronounced in urban than in semi-rural/rural areas.

In relation to phenology, there are not many specific studies of the *Fraxinus* species, in spite of the close relationship between aerobiological studies and the global climate change effects on plants (Bruns et al. 2013; Galán et al. 2016; Carter et al. 2017) and fertilization (Bochenek and Eriksen 2011). One study for one year was developed in Mar del Plata, Argentina (Latorre and Bianchi 1998), and showed that floral phenophase development is not always synchronous between trees and that differences between species may be relevant. Thus, it was observed that *Fraxinus* pollen was recorded over a longer period after the *F. excelsior* flowering season had ended. A study performed in Austria over 22 years including *Fraxinus excelsior* (Bortenschlager and Bortenschlager 2005) showed that the flowering period started earlier and lasted longer and that the peak values in pollen production had increased.

This study aims to analyse the relationships between *Fraxinus* airborne pollen trends for a period of 22 years of monitoring with the distribution of pollen sources surrounding the sampler taking into account wind direction and phenology.

2 Materials and methods

2.1 Sampling site

This study was conducted in Badajoz, a city in southwestern Spain, with approximately 149,949 inhabitants (NSI 2016). The city is 184 m above sea level and is crossed by the Guadiana River with the Gévorá River as effluent close to the city (Fig. 1). Irrigated crops (cornfields, fruit trees and other vegetables) and dried crops (wheat and oats) occur in the surrounding environment. Natural vegetation in the region includes Mediterranean forests of holm oak and cork oak, or their use as *dehesa* for livestock, and shrubs, grasses and other herbaceous plants such as *Cistaceae* sp,

Amaranthaceae sp., *Echium plantagineum* and *Plantago lanceolata*.

2.2 Planes of tree location

Fraxinus trees were explored during the period of this study in a circle 500 m in diameter surrounding the sampler, counted and geo-referenced on a map. They were counted separately according to sections of 30 degrees surrounding the spore trap. This area was selected because most of the trees surrounding the spore trap were in this area, and some previous studies had also been conducted in this area (Bricchi et al. 2000; Maya-Manzano et al. 2017a, b).

2.3 Aerobiological monitoring

Pollen grains were collected using spore traps (Hirst 1952). A trap was situated at 6 m height on the terrace of the Agricultural Engineering School of the University of Extremadura (38°53' N, 6°58' W). Standardized data management procedures for capturing and counting airborne pollen were used according to the Spanish Aerobiology Network (REA) (Galán et al. 2007) and the considerations of Tormo-Molina et al. (1996a, b, 2013). Pollen concentrations were expressed as the number of pollen grains per cubic metre of air (pollen grains m⁻³). The main pollen seasons (MPS) of *Fraxinus* were defined using the 90% method, whereby the season started when 5% of the total annual catch was achieved and ended when 95% was reached (Nilsson and Persson 1981). The period for calculation was November 1 to February 28, 121 days (2015–2016) and 120 days (2016–2017). The characteristics of the *Fraxinus* pollen season were described by the start and end dates of the airborne pollen season, the duration (number of days), the date and value of the maximum daily mean *Fraxinus* pollen concentration, and the sum of the daily average airborne *Fraxinus* pollen concentrations recorded during the season (Seasonal Pollen Integral—SPI) (Galán et al. 2017). The pollen-monitoring period was 1993–2016. There were no data for two periods (1994–1995 and 2001–2002) because the sampler did not work properly. During three additional periods, some days were lost; in this case, these periods were not used for MPS analysis.

Fig. 1 Ash tree distribution (red circles), with trees phenologically monitored (yellow squares) and distributed surrounding the spore trap (black star) within a circle of 500 m diameter divided every 30 degrees (see Table 2). Spore trap—EIA (black star) and university campus—UEx (blue star) in Badajoz

2.4 Meteorological data

The region has a Mediterranean climate affected by a degree of continentality, with an annual mean temperature for Badajoz of 17.1 °C. The highest temperatures occur during the summer, mainly during July and August. Average total annual precipitation reaches 447 mm. Prevailing winds are from the west in a region that has nearly flat topography (Silva-Palacios et al. 2007).

Meteorological data were provided by the National Meteorology Agency (AEMET) from a meteorological station at 38° 53' 15'' N, 7° 0' 29'' W, 3.75 km from the aerobiological sampler. For two periods (2015–2016 and 2016–2017), a portable meteorological station, two metres from the sampler, was used (Delta-T WS-GP1). The portable meteorological station data were recorded every 10 min, including temperature (°C), precipitation (mm), relative humidity (%), wind speed (m s⁻¹) and wind direction (degrees). To process wind direction data, 10-min values were transformed into 30° sections (12 values); for hourly and daily values, a statistical mode value was used; and for the remaining meteorological parameters, an average value was used except for precipitation, in which a sum was calculated.

2.5 Phenological observations

The phenology of pollen shedding was studied in 10 specimens over two periods (2015–2016 and 2016–2017). Five trees were selected surrounding the pollen station (EIA) and five 4 km away (UEx). Selected trees appear as yellow-coloured dots in Fig. 1. Observations were taken with a frequency of 3–4 days on average.

Phenological phases were recorded according to the BBCH code (Biologische Bundesanstalt, BUNDessortenamt, Chemische Industrie). This is an internationally recognized standard in the agricultural sector that classifies plant growth phases according to a standardized system (Meier 2001). For each tree,

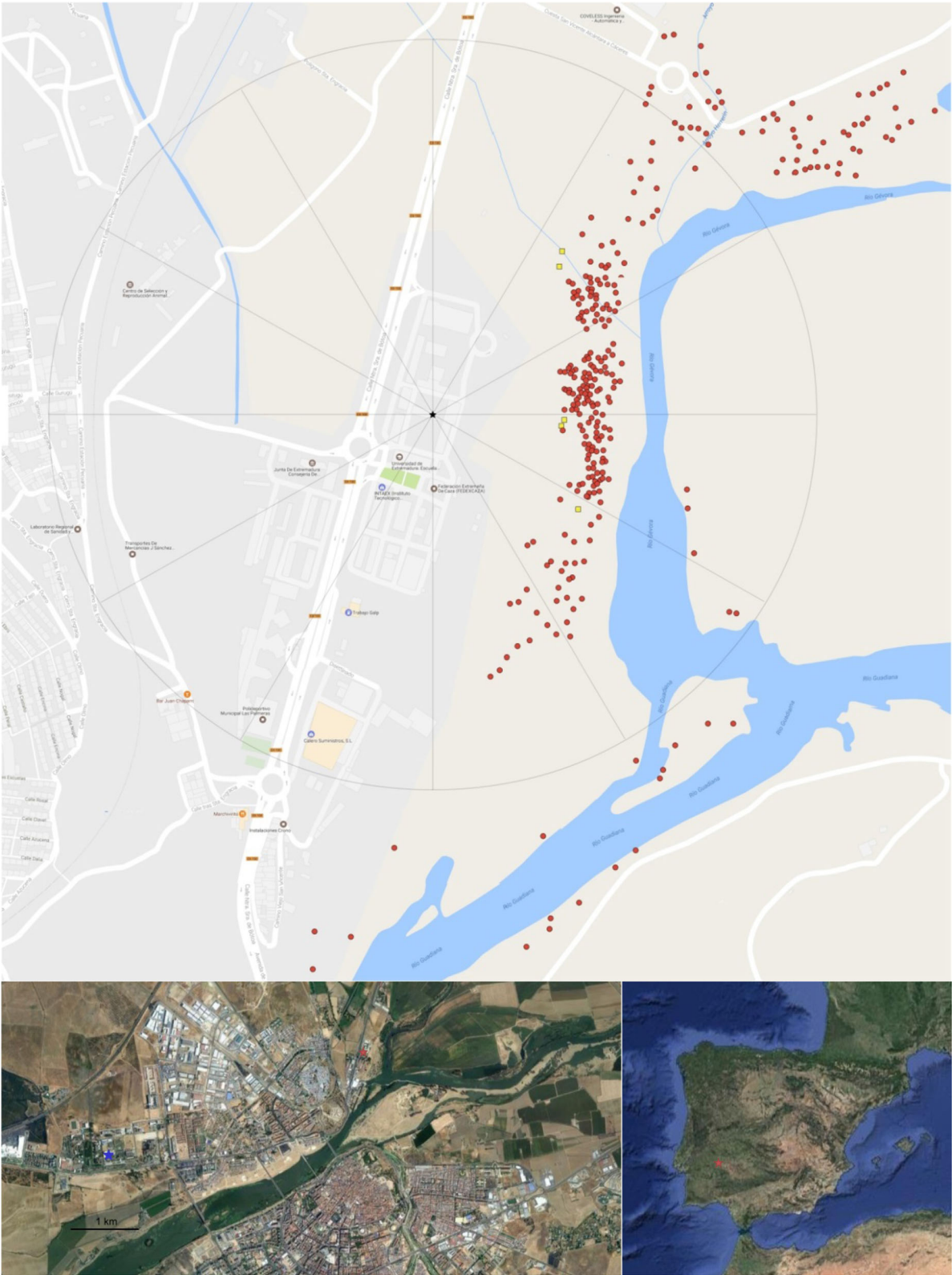


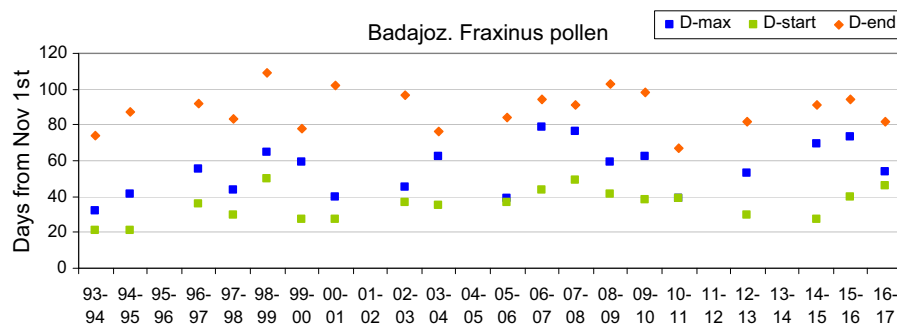
Table 1 Number of ash trees within a circle of 500 m diameter from the spore trap every 30 degrees (see Fig. 1)

Grades	Trees	Grades	Trees
0–29	0	180–209	0
30–59	69	210–239	0
60–89	78	240–269	0
90–119	56	270–299	0
120–149	27	300–329	0
150–179	11	330–359	0

pollen shedding was mechanically tested over 10 branches at 1.5–2 m height that were touched or shaken only on sunny days and with calm winds at noon. The percentage of pollen shedding was recorded from the beginning of flowering with 10% open flowers (BF, BBCH 61) up to full flowering (FF, BBCH 65, general flowering or full blossom), when at least approximately 50% of the flowers were open.

Table 2 Characteristics of the main pollen season (MPS) of *Fraxinus* pollen grains (grains m⁻³). Ave (average daily concentration), max (maximum daily concentration), day max (maximum peak of day of the year), day start (day start of the MPS), day end (day end of the MPS), length (duration of the MPS), seasonal pollen integral (SPI)

	Ave	Max	Day max	Day start	Day end	Length	SPI
93–94	4.4	52	3–12	22–11	14–1	53	530
94–95	5.8	63	12–12	22–11	27–1	66	701
96–97	4.4	69	26–12	7–12	1–2	56	532
97–98	3.2	42	15–12	1–12	23–1	53	380
98–99	6.0	57	5–1	21–12	18–2	59	719
99–00	6.9	73	30–12	28–11	18–1	51	829
00–01	4.6	51	11–12	28–11	11–2	75	556
02–03	4.0	109	16–12	8–12	6–2	60	480
03–04	9.4	130	2–1	6–12	16–1	41	1141
05–06	23.9	129	10–12	8–12	24–1	47	2868
06–07	3.6	40	19–1	15–12	3–2	50	427
07–08	5.9	78	16–1	20–12	31–1	42	709
08–09	5.8	125	30–12	12–12	12–2	62	690
09–10	2.2	51	2–1	9–12	7–2	60	262
10–11	7.8	135	10–12	10–12	7–1	28	937
12–13	2.2	32	24–12	1–12	22–1	52	265
14–15	7.3	48	9–1	28–11	31–1	64	878
15–16	5.8	48	13–1	11–12	3–2	54	700
16–17	23.0	267	25–12	17–12	22–1	36	2760

**Fig. 2** MPS values of start, maximum and end of the period studied

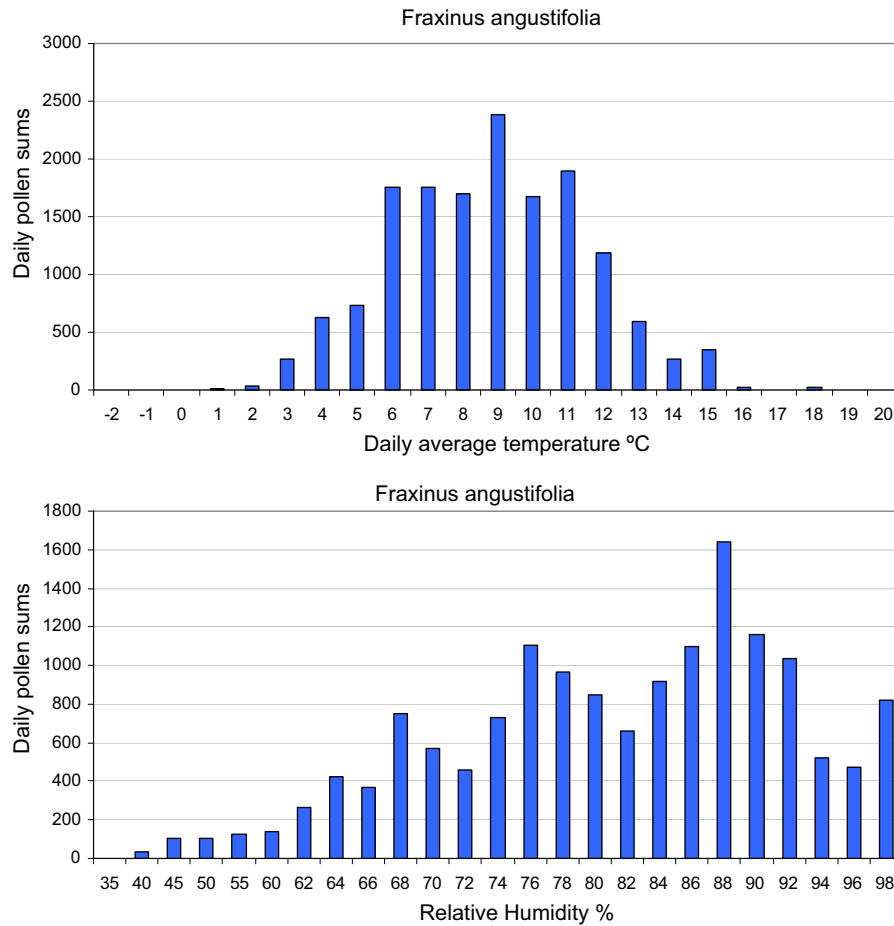


Fig. 3 Daily sums of pollen concentrations for average temperature and relative humidity

2.6 Statistical analysis

Daily data for the whole study period were used ($n = 2372$) together. A Spearman correlation test was used to analyse associations between pollen concentration and meteorological parameters (air temperature, precipitation, relative humidity and wind speed). For the last two years, more in-depth analysis using the hourly data was conducted ($n = 5784$). Statistical analysis was performed using the SPSS 15.0 statistical package. To analyse at what temperature most pollen was recorded, a graphical analysis was performed summing the daily pollen concentration for each average temperature. Data from the last two periods (2015–16 and 2016–17) were analysed in more detail using hourly data, and a graphical analysis comparing the sums of daily pollen concentration according to

temperature, relative humidity and wind direction for each of the 30 degrees was completed.

3 Results

Figure 1 shows the spore trap location and distribution of the surrounding narrow-leaved ash trees. A total of 241 trees were counted within a circle 500 m in diameter surrounding the spore trap. Most of the trees were towards the east (Table 1).

3.1 General data

Table 2 shows the average values of the MPS. Average concentration ranged from 2.2–9.4 grains m^{-3} , except for the winters of 2005–2006 and 2016–2017 with values of 23.9 and 23.0 pollen grains

Fig. 4 Daily data for the last two periods of 2015–2016 and 2016–2017: pollen concentration (pollen grains m^{-3}), temperature ($^{\circ}\text{C}$), precipitation (mm) and pollination phenology (%) for the two areas studied (EIA Agrarian Engineering School and UEx Campus University of Extremadura)

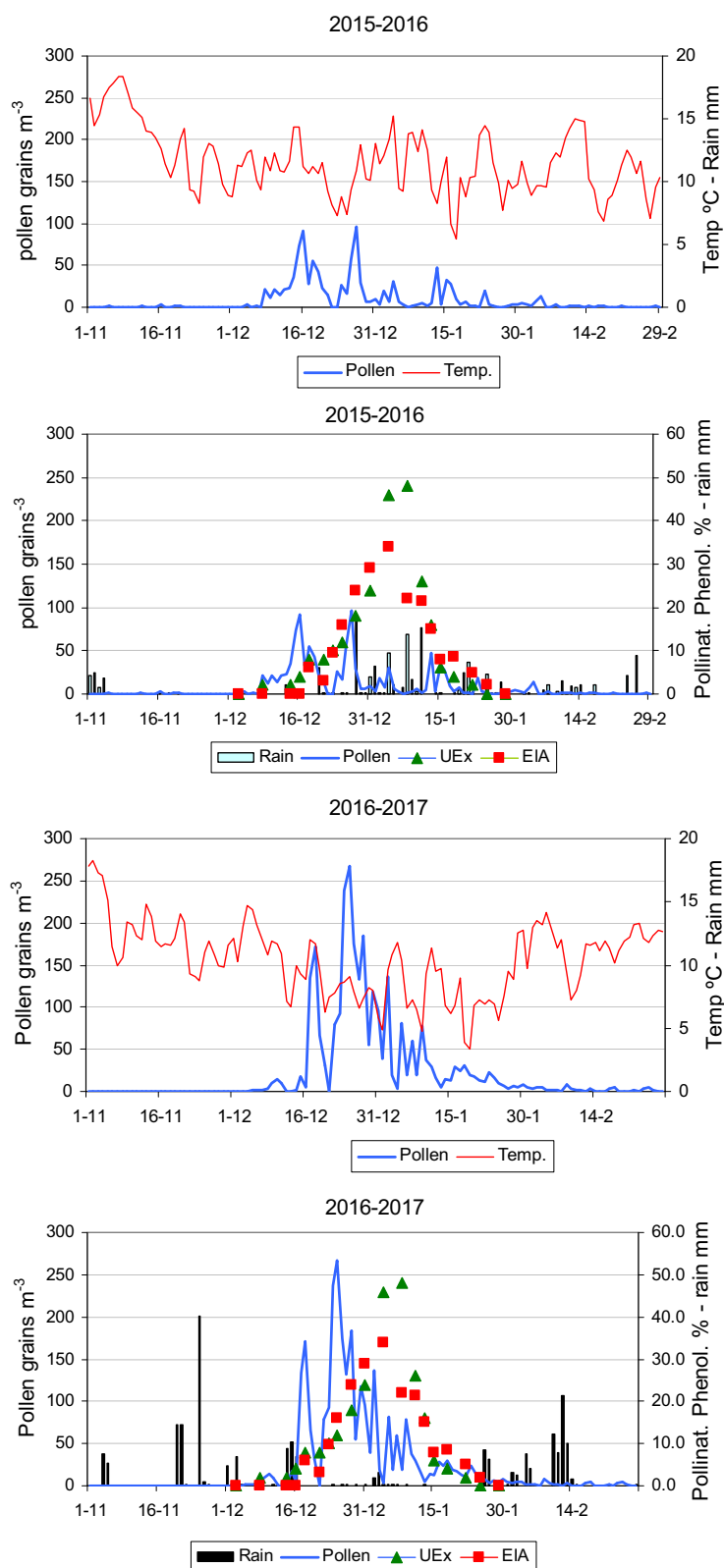
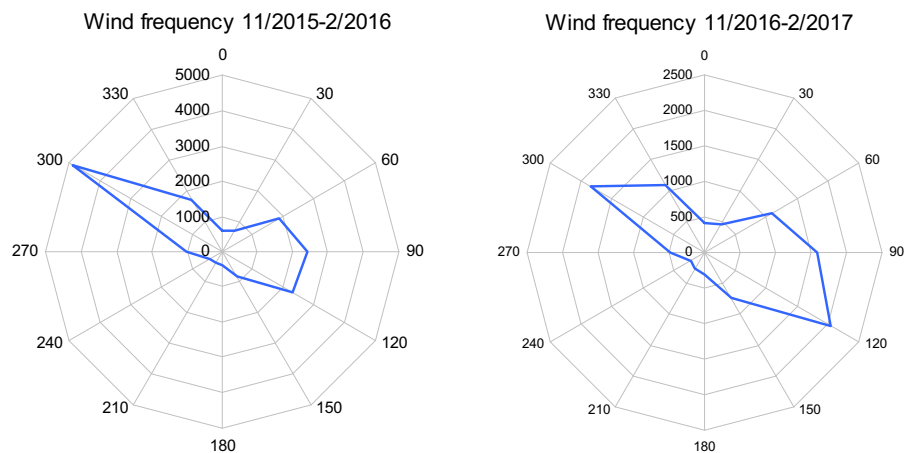


Fig. 5 Wind frequency from the period studied calculated from records every 10 min ($n = 17,424$ left, $n = 17,280$ right)



m^{-3} , respectively. The peak of maximum concentration exceeded 100 pollen grains m^{-3} during six periods, with the highest value being 267 pollen grains m^{-3} (25/12/2017). The MPS season lasted 53 days on average, ranging from 28 to 75 days; the MPS start ranged from 22/11 to 21/12, and the MPS end ranged from 14/1 to 18/2. No trend was found within the MPS (Fig. 2).

3.2 Daily data

Daily *Fraxinus* pollen concentration showed a statistically significant negative correlation with temperature ($T_{\min}$ $r = -0.190$ $p < 0.001$, $T_{\max}$ $r = -0.204$ $p < 0.001$, T_{med} $r = -0.229$), precipitation ($r = -0.079$ $p < 0.001$) and wind speed ($r = -0.075$ $p < 0.001$). There was also a positive significant correlation with relative humidity ($r = 0.073$ $p < 0.001$). Most pollen was recorded at an average temperature of 9 °C and with a relative humidity of 88% (Fig. 3).

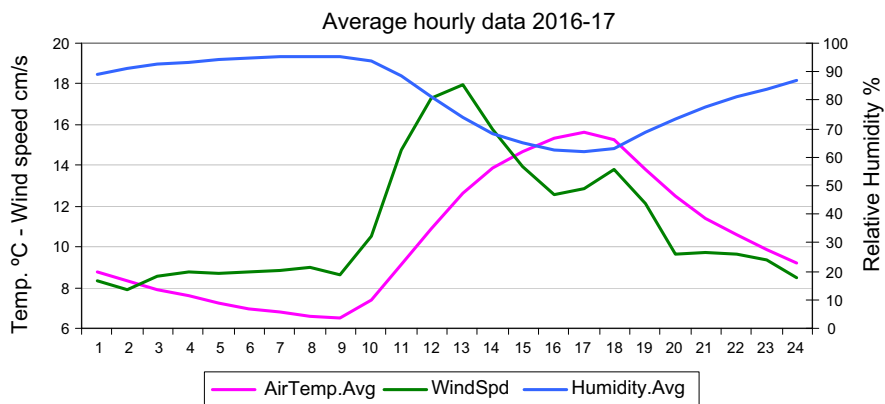
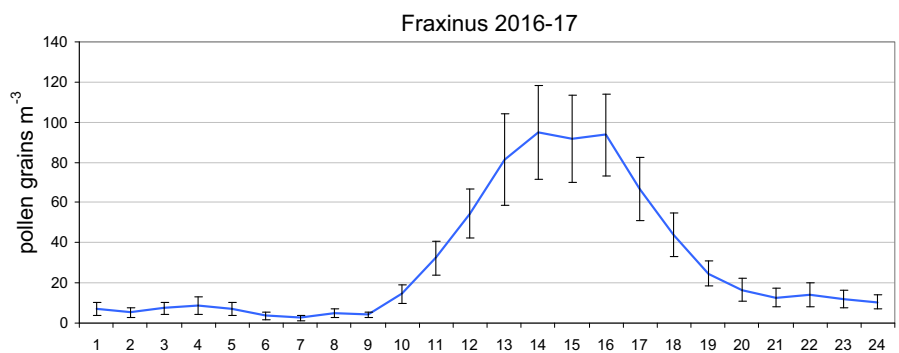
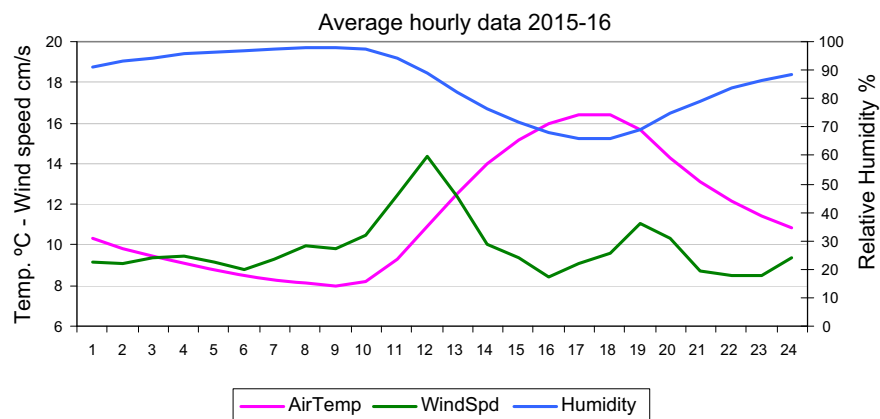
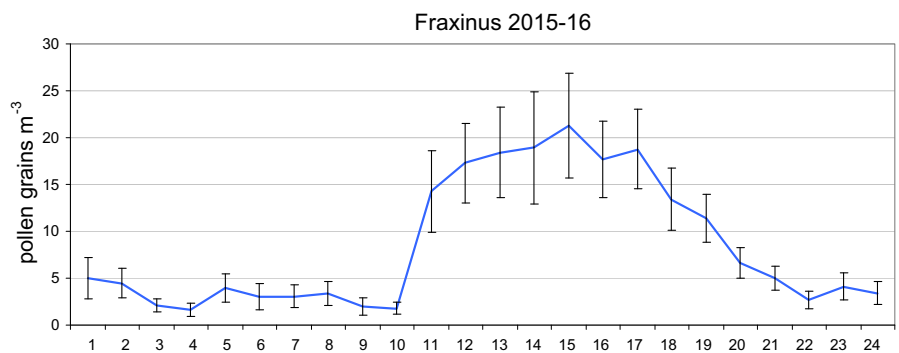
Figure 4 shows daily *Fraxinus* concentrations for the two periods (2015–2016 and 2016–2017), including pollen concentration, temperature, precipitation and pollination phenological observations. The pollen peak reached 96 pollen grains m^{-3} (27/12/2015) and 238 pollen grains m^{-3} (24/12/2016) during the two periods. During the first period, precipitation accumulated to 155 mm (54 days) and occurred mainly during December and January; for the second period, precipitation accumulated to 216 mm (46 days) and occurred mainly during November and February.

Pollination phenology started at the same time that pollen was recorded, and after the end of pollen dispersal, it was recorded at a very low concentration. Both phenologically studied locations showed quite similar patterns although peaks did not show coincidence with airborne pollen occurrence.

3.3 Hourly data

Hourly *Fraxinus* concentration for the last two periods showed a statistically negative significant correlation with relative humidity ($r = -0.210$ $p < 0.001$) and precipitation ($r = -0.079$ $p < 0.001$) and a significant positive correlation with temperature ($r = 0.058$ $p < 0.001$) and wind speed ($r = 0.059$ $p < 0.001$). In relation to wind directions, winds were predominantly from the WNW and then from the ESE, and both periods were quite similar in this regard (Fig. 5).

Figure 6 shows hourly concentration for the last two periods. The highest values appeared between 14:00 and 16:00, and the lowest appeared between 22:00 and 09:00. The increase during the morning is more pronounced than the decrease during the evening. By contrast, sums of hourly pollen concentration depending on wind direction according to 30-degree sections were calculated (Fig. 7). Most of the airborne pollen was recorded when the wind blew from the 90–120 degrees, which is coincident with location of the majority of trees.



◀ **Fig. 6** Hourly data for *Fraxinus* pollen concentration, including error bars and average meteorological parameters

4 Discussion

Narrow-leaved ash (*Fraxinus angustifolia*) is the most precocious tree species pollinating during the year, as it sheds pollen at the beginning of winter. In our study, pollen reached peak concentrations higher than those observed in Seville, Spain (Candau et al. 1994), but lower than La Plata, Argentina (Nitui and Mallo 2002; Nitui et al. 2003), and Zurich, Switzerland (Peeters 1998). Nevertheless, in the first case, a Cour sampler was used, and in the second, other species were present. Our results were similar to those of Kasprzyk et al. (2004) in Poland and Bicakci et al. (2017) in Turkey.

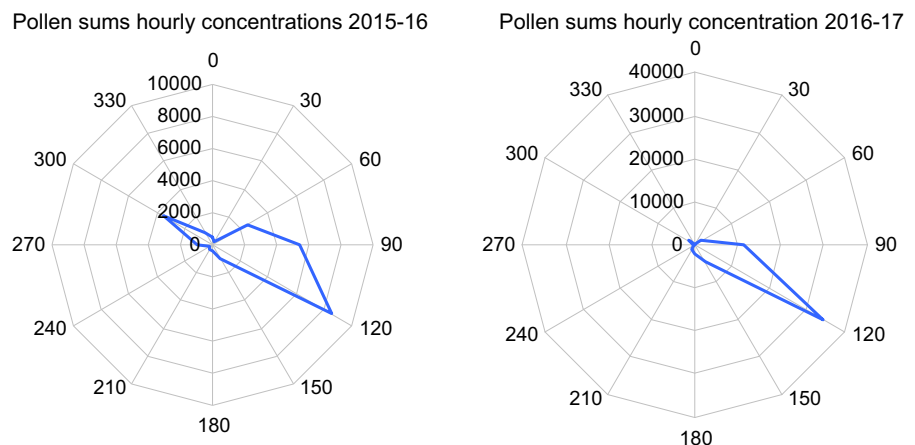
In the western Mediterranean region, narrow-leaved ash trees are common along river banks but sometimes are cultivated as ornamentals. In the first case, only when natural vegetation is conserved do these trees appear. This means that water stress should not be a factor that affects pollination, and it may depend largely on temperature. Our results showed that the best meteorological parameters linked to high pollen shedding were when daily temperature reached 9 °C. We observed a coincidence of the main pollination with the period of the year with average temperature ranges between 6° and 11° C (December–January).

The pollen season's start has been related to frost periods. For *Fraxinus excelsior* (Peeters 1998), this season occurs when the absolute minimum temperature is less than or equal to −2 °C. Comparable results

were obtained by Jato et al. (2004) in the north-western Iberian Peninsula: the accumulated cold in November determined the heat requirements and the start of pollen season and maximum pollen concentrations were captured during the end of January or the beginning of February. Nevertheless, these results do not coincide with those found in our study of *Fraxinus angustifolia*. The MPS start occurred between the end of November and the beginning of December, when the lowest temperatures had not yet been reached, similar to the conclusions found by Bicakci et al. (2017) in Turkey. These results may suggest that the photoperiodic signal that activates flowering in *Fraxinus* is close to the flowering date inasmuch as the effect of cold is not clear.

No statistically significant trends were observed in the MPS during the studied years; this may be in consonance with Kasprzyk et al. (2004), whose results did not find latitudinal differences in the main pollen season in Poland, although Ziello et al. (2012) found a pollen concentration increase in several places in Europe. This is because these trees are more independent of water stress, as they always grow in humid environments. By contrast, a study in Spain showed positive trends for *Fraxinus* airborne pollen in some places (Galán et al. 2016), and in the USA, *Fraxinus americana* showed a marked influence in leaf emergence phenology in relation to extremely warm years (Carter et al. 2017). However, we obtained a significant correlation with meteorological parameters, although with different signs depending on the use of daily or hourly data. Daily data showed a negative correlation with temperature and a positive correlation with relative humidity, as obtained by Calderón-

Fig. 7 Pollen sums from hourly concentration according to wind direction



Ezquerro et al. (2016), but hourly data showed the opposite. These results may be because pollination phenology occurs when daily temperatures are decreasing, and this is shown by the correlation with daily data. Nevertheless, during the pollination period, the pollen is really shedding and flying when the hourly temperature reaches approximately 12 °C, around noon. Similar results were also obtained by Ščevková et al. (2015) and Calderón-Ezquerro et al. (2016). In addition, this is time when winds showed their highest values (Fig. 6). These paradoxical results are a consequence of the time-level analysis because seasonal phenology is observed by daily data, but pollination and pollen records are observed as hourly phenomena dependent on hourly environmental conditions. We have to emphasize that hourly pollen distribution showed a neat pattern over the two last periods analysed that was similar to the hourly temperature distribution, which contrasts with the results of Simoleit et al. (2016) in Germany and Pérez-Badia et al. (2010) in Spain (Toledo) but is in accordance with Puc (2012) in Poland, Ščevková et al. (2015) in Slovakia, and Nitiu and Mallo (2002) and Nitiu et al. (2003) in Argentina.

The analysis of pollen source location and pollen records according to wind direction (Figs. 5 and 7) reflected a close relation between ash tree distribution surrounding spore traps and wind transporting airborne pollen. Although winds are predominantly in a NW–SE direction, practically all *Fraxinus* pollen grains were recorded when the wind blew from the SE (in spite of being in an E or NE direction where ash trees are more abundant, Fig. 1). These results support the relevance of a close-source location and winds transporting the pollen in the aerobiological records using Hirst spore traps (Silva-Palacios et al. 2007; Fernández-Rodríguez et al. 2014). Another factor can be deduced considering the predominant wind direction, the location of the trap and the pollen source distribution. There are no sources for this pollen type when the wind blows from the W. This could lead to underestimation of the airborne *Fraxinus* pollen concentrations that actually are present in the atmosphere, as the spore trap location with respect to the predominant wind direction is an essential point to be considered in aerobiological studies (Maya-Manzano et al. 2017a, b).

Both phenologically analysed locations (natural and ornamental river banks) showed similar results.

This may be because both showed an absence of water stress, as the ornamental locations were artificially watered even during summer. Correspondence between phenology and pollen occurrence showed a similar start but an earlier ending for phenology as compared to that of the pollen records, similar to the results obtained by Bortenschlager and Bortenschlager (2005). Nevertheless, pollen peaks did not show correspondence with phenology peaks. With respect to the two last periods more comprehensively analysed, in the first, this would be a consequence of precipitation distribution, which could have resulted in air cleansing, but in the second period, there is a clear delay of pollination phenology of approximately 10–15 days with respect to pollen occurrence. It could be alleged that these results for pollen records reflect long-distance pollen transport; however, our results from source location analysis showed that close trees within a 500-m-diameter circle surrounding the spore trap may have been responsible for the most pollen recorded. This may be a consequence of the fact that phenology records were obtained for branches at 1.5–2 m height to obtain accurate results, and the top branches escaped recording. Another explanation of the results may be the distribution of phenologically observed trees because not all were in the main wind direction. We observed that the top branches of the tree were not exactly synchronized with the bottom branches. Differences in phenology have been found among trees depending on age (Augspurger and Bartlett 2003) or even among branches of the same tree, especially in deciduous trees (Osada et al. 2005). Moreover, in Argentina, a synchronicity between the phenology of trees was not found (Latorre and Bianchi 1998). However, this issue requires further study to assess this possible explanation. To these variations, variations may be added in relation to altitude and latitude (Damialis et al. 2011, 2017). We should have also considered that not all of the whole pollen grains that were recorded came from sources located in the direction from which the prevailing winds blow. Wind dynamics are a complex process, and we obtained limited information to produce definitive conclusions with only one pollen record point.

5 Conclusions

For narrow-leaved ash (*Fraxinus angustifolia*), no statistically significant trend in pollination was found, and this may be because their ecology is more independent of water stress. Although seasonal analysis showed a negative correlation between pollination and temperature, hourly data showed a clear direct dependence on temperature for pollen shedding. It has been observed that pollination in these trees occurs close to the cooler period of the year but during the warmer hours of those days. We showed that the analysis of pollen source distribution and wind origin is relevant to interpreting *Fraxinus angustifolia* airborne pollen occurrence. Nevertheless, the phenology of pollination may not be in accordance with the pollen peaks and pollination peaks, and this may be related to the methodology of phenology recording.

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