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Maria Dimou. Comparison of phenological, aerobiological and melissopalynological patterns of Olea europaea. Apidologie, 2011, 43 (1), pp.103-112. 10.1007/s13592-011-0100-2. hal-01003523

# HAL Id: hal-01003523 https://hal.science/hal-01003523

Submitted on 11 May 2020

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# Comparison of phenological, aerobiological and melissopalynological patterns of *Olea europaea*

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Received 30 October 2010 - Revised 19 January 2011 - Accepted 3 July 2011

**Abstract** – In the last few years, many studies have used airborne pollen to record the flowering season of plants. However, the airborne pollen found in traps does not always originate from or reflect the flowering period of the taxa from the studied surroundings. This study compared aerobiological, melissopalynological and flowering phenological data obtained from olive trees. The results of the study suggest that pollen loads collected by honeybees can be used as an alternative method to airborne pollen to record the flowering phenological phases and to provide accurate results.

aerobiology / melissopalynology / phenology / pollen season / pollen loads / Apis mellifera

# 1. INTRODUCTION

Phenology studies the periodical rhythms of the biological events and their relationships with the environment, especially the climate. As the timing of phenological events and especially flowering is strongly affected by meteorological factors, it has been observed that plant phenological responses correlate with climatic change (Sparks et al., 2000; Kramer et al., 2000; Defila and Clot, 2001; Menzel, 2000; Chmielewsk et al., 2005; Estrella et al., 2006). Moreover, the knowledge of the phenological stages of the plants, such as the flowering time, can be an important tool for agricultural economics (e.g. pesticides and fertilizer applications, choice of varieties of plants, crop yield) (Fernandez-Mensaque et al., 1998; Censi and Ceschia, 2000; Moriondo et al., 2001; García-Mozo et al., 2008).

Pollen release during flowering can be recorded at a regional scale with the use of airborne pollen traps (Osborne et al., 2000; Jato et al., 2002b; Aguilera and Valenzuela, 2009). Recently, a number of studies have also used airborne pollen data as an indicator of climatic change or to create forecasting models for agricultural applications (Frenguelli, 1998; Newnham, 1999; Osborne et al., 2000; Emberlin et al., 2002; Van Vliet et al., 2002; Clot, 2003; Orlandi et al., 2005; Ribeiro et al., 2006; Galán et al., 2008; García-Mozo et al., 2008).

However, this method has some drawbacks. Airborne pollen is very mobile, due to its size and structure. The long-distance transport of pollen is well known, and several studies have demonstrated the ability of airborne pollen to transfer hundreds of kilometres away (Cambon et al., 1992; Cabezudo et al., 1997; Latorre, 1997; Jato et al., 2002b; Kasprzyk, 2003; Rousseau et al., 2003; Van de Water et al., 2003; Lorenzo et al., 2006; Ranta et al., 2006; Estrella et al., 2006). Therefore, pollen found in airborne pollen

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traps does not necessarily reflect the pollen from the studied surroundings.

Moreover, the end of the main atmospheric pollen season can also be influenced by the presence of pollen resuspended from the ground once flowering has finished (Dopazo et al., 2003; Hidalgo et al., 2003; Waisel et al., 2008). In general, aerobiological observations about the pollen season are strongly influenced by the taxon, the location and the large-scale weather conditions (Estrella et al., 2006; Jato et al., 2006). Thus, phenological observations should be made in order to define the aerobiological pollen season according to the local flowering, especially when airborne pollen is used as bioindicator of flowering phenophase in climate changes investigations (Fornaciari et al., 2000; Garcia-Mozo et al., 2009; Jato et al., 2006).

The olive tree is one of the most important cultivated and uncultivated plants in the Mediterranean countries. The flowering period of *Olea europaea* L. is strongly correlated to the yearly temperature variations (Chuine et al., 1998; Bonofiglio et al., 2009) and thus, several authors have proposed and used this plant as an indicator for climatic change (Osborne et al., 2000; Orlandi et al., 2005).

Pollen traps have been used in several studies to record the diet of the bees (Severson and Parry, 1981; Pearson and Braiden, 1990; Telleria, 1993; Coffey and Breen, 1997; Webby, 2004; Andrada and Telleria, 2005; Dimou and Thrasyvoulou, 2007). Yet, few studies have compared the honeybee collected pollen to the flowering phenology of the plants in an area (Andrada and Telleria, 2005; Dimou and Thrasyvoulou, 2007).

The aim of this research was to analyse and compare the effectiveness of the melissopalynological records as an indicator of the phenological flowering phases and compare the results to those that the aerobiological records provide. To achieve this goal, we compared the results among airborne pollen trap, pollen traps attached to hives of honeybees, and flowering phenological data obtained from olive trees.

Although honeybees collect pollen mainly from entomophilous plants, they also collect pollen from several anemophilous plants in significant amounts (Severson and Parry, 1981; Coffey and Breen, 1997; Dimou and Thrasyvoulou, 2007). Olea pollen has been recorded in many apicultural and aerobiological studies in the Mediterranean (Ricciardelli d'Albore and Battaglini Bernardini, 1978; Diaz-Losada et al., 1998; Grau et al., 2000; Galán et al., 2001; Sa-Otero et al., 2002; Terrab et al., 2003, 2004). Since both aerobiological and melissopalynological studies in Thessaloniki, Greece have demonstrated that olea is one of the dominant pollen types (Gioulekas et al., 2004a, b; Damialis et al., 2007; Dimou and Thrasyvoulou, 2007), this taxon was considered as the most suitable for the purpose of this study.

# 2. MATERIALS AND METHODS

#### 2.1. Study area

Flowering phenological and pollen data were collected at the farm of the Aristotle University of Thessaloniki in Greece. The olive orchard (2.8 ha) was located near the traps, approximately 0.5 km away from the apiary and 1 km from the airborne pollen trap. The trees of the orchard belonged to five different local varieties with the majority of the trees belonging to a single variety.

## 2.2. Phenological data

Measurements of flowering phenology were carried out daily from May to June in 2008 and 2009, when the flowering period of *O. europaea* in the area occurs (Liccardi et al., 1996). Six trees that belonged to the most cultivated variety in the area and four trees that belonged to the remaining four varieties were randomly selected. From at least 20 randomly chosen branches of each tree, the numbers of closed, open and fallen flowers were counted (Latorre, 1997).

#### 2.3. Honeybee pollen collection

Four pollen traps were fitted to the entrance of four respective hives of *Apis mellifera* L. from March to June in 2008 and 2009. The whole amount of pollen was collected daily, cleaned of debris by hand, dried and weighed. Then, 10% of the total amount of each trap was sampled and the pollen loads were separated according to their colour (Dimou et al., 2006). At least two representative pellets of each colour fraction were mounded in glycerine jelly without acetolysis in order to identify the botanical origin of each colour. In each slide, we counted at least 300 pollen grains to verify the homogeneity of each colour fraction (Percival, 1947; Pearson and Braiden, 1990). Pollen loads from olea were polled together in order to evaluate their contribution, and the results were expressed as a percentage of the total amount.

## 2.4. Airborne pollen collection

One airborne pollen Burkard 7-day recording volumetric trap was placed near the apiary (approximate distance 0.7 km), 12 m above the ground, from March to July in 2008 and 2009. Daily pollen grains were identified and counted by horizontal continuous dragging, recording at least 20% of the slide area under a light microscope at a magnification of  $\times$ 400 according to the protocol proposed by the Italian Association of Aerobiology (Mandrioli, 1994). Counts were expressed as mean daily pollen concentrations (number of pollen grains per cubic metre of air).

## 2.5. Meteorological parameters

Daily records of mean air temperature (degree Celsius), rainfall (millimetres), relative humidity (percent) and wind speed (kilometres per hour) were obtained from the Aristotle University of Thessaloniki Meteorological Station established at the same area.

#### 2.6. Statistical analysis

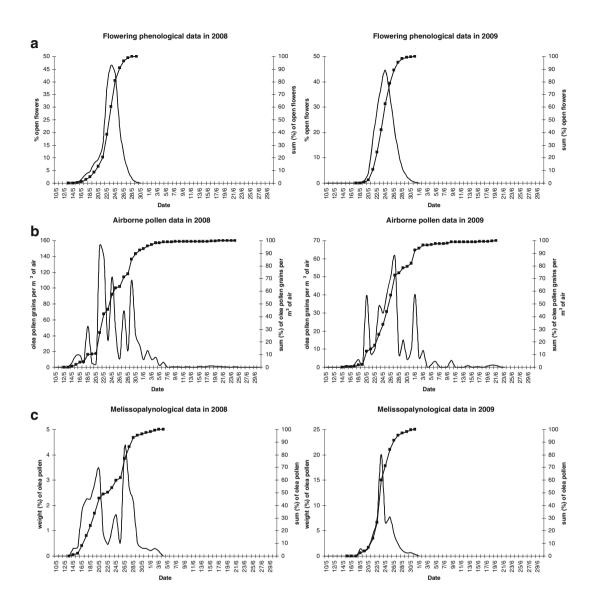
Non-parametric Spearman's correlation coefficients were calculated among the phenological, aerobiological and melissopalynological data as well as the different meteorological parameters. Non-parametric tests were preferred because the data were not normally distributed in all cases. The association among the methods concerning the start, peak and end day of the flowering period was assessed using the chi-square test. The analyses were carried out using SPSS v.16.0 (SPSS, Inc., Chicago, IL) for Windows (Microsoft Corporation, Redmond, WA). The significance level of all the statistical tests was set at  $\alpha$ =0.05.

# 3. RESULTS

The flowering of the olive trees started in the middle of May and ended at the end of the same month in both years (Figure 1a). In particular, in 2008, the first flowers opened on May 14 and the last ones were observed on May 29. In 2009, the flowering period was 2 days shorter. The first open flowers were observed on May 18 and the last ones on May 31. The peak was on May 23 in 2008 and on May 24 in 2009 (Table I). Meteorological parameters did not have a statistically significant influence on the flowering of the olive trees in 2008 (minimum observed P=0.057). On the contrary, there was statistically significant correlation between the flowering rate and the temperature as well as the relative humidity in 2009 (P=0.005, Spearman correlation=0.701 and P=0.005, Spearman correlation = -0.701, respectively).

O. europaea pollen grains were detected in the airborne pollen trap from the middle of May until the end of June in both years (Figure 1b). Compared to the flowering season, the olea pollen in the air was located 1 day earlier in 2008 and 3 days earlier in 2009. The greatest concentration in the air was located in the second half of May for both years. The pollen curves were characterized by several peaks. The main one was observed on May 21 in 2008. By that time, 27% of the total annual olea pollen had been recorded. Accordingly, the main peak for 2009 was observed on May 27, at which point 72% of the total annual olea pollen had been recorded. During June, the pollen grains were located sporadically. The 95% and 99% of the total annual pollen amount were reached on June 2 and 6 in 2008 and on June 3 and 16 in 2009 (Figure 1b). Meteorological parameters did not have a statistically significant influence on the concentration of olea pollen in the air during the period of flowering in either of the 2 years ( $P_{\min}=0.103$ ).

Honeybees collected olea pollen loads from the middle of May until the first days of June in both years (Figure 1c). The first pollen loads



**Figure 1.** Daily phenological, aerobiological and melissopalynological data of *O. europaea* in 2008 and 2009: **a** number (percent) of bloomed flowers (*thin solid line*) and sum (percent) of bloomed flowers (*dotted line*); **b** number of pollen grains per cubic metre of air (*thin solid line*) and sum (percent) of pollen grains per cubic metre of air (*dotted line*); **c** weight (percent) of pollen loads collected by honeybees (thin solid line) and sum (percent) of pollen load amount (*dotted line*).

were collected on May 14 in 2008, the same day in which the first open flowers were observed at the olive trees. In 2009, the first olea pollen loads were collected on May 16, 2 days earlier than the first open flowers. The main concentration was observed on May 20 and 26 in 2008. By these time points, 46% and 76% of the total annual pollen had been collected, respectively. Accordingly, the main concentration for 2009 was observed on May 23, at which point 60% of the total annual pollen had been collected (Figure 1c). Olea pollen collection stopped

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**Table I.** Phenological, aerobiological and melissopalynological data presenting the sum (percent) and confidence intervals regarding the bloomed flowers, the pollen grains per cubic metre of air and the amount of pollen loads at the start, peak and end day of the flowering season of *O. europaea* in 2008 and 2009.

Date	Phenological data		Aerobiological data			Melissopalynological data		
	Sum (%) of open flowers	95% CI	sum (%) of airborne pollen	95% CI	P value	Sum (%) of pollen loads	95% CI	P value
2008								
Start (14/5)	0.2	0-0.3	0.6	0.1-1.1	$0.032^{a}$	0.3	0.1 - 0.4	0.089
Peak (23/5)	60.1	58.1-61.9	45.4	42.9-47.9	< 0.001 <sup>a</sup>	54.3	52.4-56.2	< 0.001 <sup>a</sup>
End (29/5)	99.9	99.8-100.0	85.1	83.5-86.7	< 0.001 <sup>a</sup>	95.6	94.3–96.9	$0.004^{a}$
2009								
Start (18/5)	0.2	0-0.3	1.8	1.3-2.3	< 0.001 <sup>a</sup>	2.4	1.6-3.2	< 0.001 <sup>a</sup>
Peak (24/5)	63.2	61.1-64.9	33.6	32.8-35.4	< 0.001 <sup>a</sup>	71.1	69.7-72.5	< 0.001 <sup>a</sup>
End (31/5)	99.9	99.8-100.0	82.2	80.7-83.7	< 0.001 <sup>a</sup>	99.9	99.7-100.0	0.398

<sup>a</sup> Statistically significant difference according to chi-square test between the phenological and the aerobiological (or melissopalynological) data ( $\alpha$ =0.05)

within 2 days from the date when the last open flower was observed. Although the total amount of olea pollen was different among the honeybee colonies, statistical analysis about the rate of pollen load collection among them showed significant correlation in all cases ( $r_{\min}=0.642$ ,  $P_{\text{max}}=0.004$ ). Meteorological parameters did not have a statistically significant influence on the pollen collection in 2008 ( $P_{\min}=0.440$ ). On the contrary, similarly to the phenological results, there were statistically significant differences between the rate of pollen load collection and the temperature and between the rate of pollen load collection and the relative humidity in 2009  $(P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.789 \text{ and } P < 0.001, \text{Spearman correlation} = 0.001, \text{Spearman c$ 0.001, Spearman correlation=-0.815, respectively). Rainfall on 22, 24 and 25 of May in 2008 seemed to have a direct effect on the foraging amount of olea pollen loads and, to a lesser extent, on airborne pollen (Figure 1); however, it was not statistically significant.

Statistical analysis between the flowering and the melissopalynological data (depicted with thin solid lines in Figure 1) showed significant correlation for both years (P<0.001, Spearman correlation=0.664 in 2008 and P<0.001, Spearman correlation=0.864 in 2009). On the contrary, a similar analysis showed that there was no significant correlation between the olea pollen grains counted in the air and the flowering phenological data ( $P_{\min}=0.073$ ) or the olea pollen grains counted in the air and the daily olea pollen collected by the honeybees ( $P_{\min}=0.220$ ) in either of the 2 years.

The comparison between the sum (percent) of open flowers that were observed at the start, peak and end day of the flowering period and the sum (percent) of olea pollen grains that were counted in the air on the same dates showed statistically significant differences in all three cases for both years (P<0.001). Similar results were also obtained from the comparison between the sum (percent) of open flowers and the sum (percent) of the amount of olea pollen loads collected by the honeybees on the specific dates. However, in this case, there was no statistically significant difference for the start day in 2008 (P=0.089) and the end day in 2009 (P=0.398) (Table I).

The average temperature during the flowering period of olive trees was  $21.6\pm2.4$ °C in 2008 and  $22.6\pm2.2$ °C in 2009. The average relative humidity was  $60.3\pm5.5\%$  in 2008 and  $62.8\pm8.4\%$  in 2009. The wind speed ranged from 0.3 to 1.0 km/h in 2008 and 0.3 to 1.5 km/h in 2009. Finally, the average rainfall was  $0.7\pm1.4$  mm in

2008 and  $0.6\pm0.2$  mm in 2009. Statistical analyses showed no significant differences between the 2 years for any of the meteorological parameters ( $P_{min}=0.200$ ).

## 4. **DISCUSSION**

The determination of the pollen season phases is an important goal in aerobiological, agricultural and ecological studies (Minero et al., 1998; Benninghoff, 1991; Latorre, 1999; Moriondo et al., 2001; Jato et al., 2002a, b, 2006, 2007; Ribeiro et al., 2007). Thus, several authors have proposed methods to define the pollen season duration or phases such as the start, peak and end date (Nilsson and Persson, 1981; Lejoly-Gabriel and Leuschner, 1983; Andersen, 1991; Spieksma et al., 1995; Jager et al., 1996; Feher and Jarai-Komlodi, 1997; Giorato et al., 2000; Sanchez-Mesa et al., 2003). However, the accuracy of these methods is strongly connected to the taxon, the location and the large-scale weather conditions (Estrella et al., 2006; Jato et al., 2006).

The first day of olea pollen presence in the air was found to be no more than 3 days earlier than the first open flowers in both years of the study, and in general, there was some correlation between the concentration of olea pollen in the air and the flowering period of the olive trees. However, this correlation was not statistically significant and several peaks were observed in the pollen curves during the flowering period.

At the peak of the flowering period, less than 50% of the annual total olea pollen in the air had been captured (45% in 2008 and 33% in 2009). Moreover, the pollen season was found to be longer in the air traps compared to the flowering phenological observations. Other studies have also demonstrated a long period of olive pollen grains in the air compared to the flowering period of the trees (Gioulekas et al., 1991; Liccardi et al., 1996; Minero and Candau, 1997; Fornaciari et al., 2000; Altintas et al., 2004; Gioulekas et al. 2004a, b). Meiffren (1988) and Fornaciari et al. (2000) have reported the ability of olea pollen to travel over middle and long distances. Indeed, the start and

end date as well as the peak day of the pollen season can be influenced by the long-range transport phenomena of olive pollen from neighbour regions (Vázquez et al., 2003; Galan et al., 2004; Galán et al., 2008). Thus, several authors point out that phenological data should also be obtained in order to specify the aerobiological pollen season with respect to the local flowering in a more accurate way (Fornaciari et al., 2000; Garcıa-Mozo et al., 2009; Jato et al., 2006).

The melissopalynological results from this study showed a statistically significant correlation between the olea pollen load collection and the flowering season of the olive trees. Although there was not always statistically significant relevance between the number of open flowers and the amount of the collected pollen loads on the key dates of the flowering season, the start (and the end) of olea pollen collection by the honeybees was no more than 2 days apart from the start (respectively end) of flowering, in both years of the measurements. Previous studies have showed that there is a timing correlation between the pollen collection by honeybees and the flowering period (Andrada and Telleria, 2005; Dimou and Thrasyvoulou, 2007). Moreover, honeybees collect food only from a restricted area around the hive (Visscher and Seeley, 1982; Ekert, 1993; Steffan-Dewenter and Tscharntke, 1999). Thus, compared to the air pollen traps, the pollen collected by traps adjusted to beehives strictly represents the taxa only from the area around the apiary.

Honeybees are broad polylectic insects (Cane and Sipes, 2006) and several authors have showed their preferences to specific taxa (Boelter and Wilson, 1984; Page et al., 1995; Pernal and Currie, 2001, 2002; Cook et al., 2003; Pankiw et al., 2002). Although honeybees collect pollen from a relative small number of taxa compared to the number of plants growing around the colony, they usually prefer to collect pollen from plants that are in large populations (Severson and Parry, 1981; Coffey and Breen, 1997; Andrada and Telleria, 2005; Dimou and Thrasyvoulou, 2007; Avni et al., 2009). Also, their preferences are constant throughout the years and depend mainly on the meteorological factors (Severson and Parry, 1981; Andrada and Telleria, 2005; Dimou et al., 2006; Dimou and Thrasyvoulou, 2007; de Novais et al., 2009).

In this study, although the total amount of olea pollen collected from each colony was different, the rate of collection among the colonies was the same. According to Dimou et al. (2006), even a small number of colonies can give sufficient information about a taxon when it appears in large populations and the annual pollen collection from this taxon exceeds 1% of the total pollen that the colony collects. The difference in the total amount of olea pollen that was collected by the honeybees between the 2 years is likely to be caused by the phenomenon of the biannual bearing of olive trees which influences the year-to-year pollen flow (Monselise and Goldschmidt, 1982). Similar observations had also been reported in a previous study in the area (Dimou et al., 2006).

Airborne pollen is due mainly to anemophilous plants, while entomophilous pollen is found in small percentages (Kasprzyk, 2004). Thus, in comparison to airborne pollen traps, the study of honeybee pollen loads could provide flowering phenological data from a large number of entomophilous plants. Moreover, the parallel study of pollen using both airborne and honeybee pollen traps could also help define more accurately the pollen season derived from aerobiological studies. Finally, bee pollen data collected for apicultural reasons could be used to determine proxy phenological response to climate change in the past, especially in areas where there is a lack of aerobiological data.

In conclusion, the results of this work showed that the study of honeybee collected pollen could be used as an alternative method to airborne pollen traps to record the flowering period of the plants. Further studies using and comparing data of several taxa in different locations and time would be useful to assess in more detail the use of melissopalynological analysis as a bioindicator of flowering phenophase in climate changes investigations to climatic modelling. Comparaison des profils phénologique, aérobiologique et mélissopalynologique d'*Olea europaea* 

Aérobiologie / mélissopalynologie / phénologie / période de pollinisation / *Apis mellifera* / pelote de pollen / olivier

Vergleich phänologischer, luftbiologischer und melissopalynologischer Muster von Olea europaea

Luftbiologie / Melissopalynologie / Phenologie / Pollensaison / Pollenladung / *Apis mellifera* 

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