



Grana

ISSN: 0017-3134 (Print) 1651-2049 (Online) Journal homepage: <https://www.tandfonline.com/loi/sgra20>

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To cite this article: Fátima Aguilera & Luis Ruiz Valenzuela (2012) Microclimatic-induced fluctuations in the flower and pollen production rate of olive trees (*Olea europaea* L.), Grana, 51:3, 228-239, DOI: [10.1080/00173134.2012.659203](https://doi.org/10.1080/00173134.2012.659203)

To link to this article: <https://doi.org/10.1080/00173134.2012.659203>



Published online: 30 Mar 2012.



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Microclimatic-induced fluctuations in the flower and pollen production rate of olive trees (*Olea europaea* L.)

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Abstract

The possible impact of altitude and the related microclimatic conditions on the total production of fruiting branches, inflorescences, flowers and pollen grains of olive trees *Olea europaea* was analysed. A total of 90 Picual cultivar trees, the most extensive olive cultivar in the Iberian Peninsula, were studied for a three-year period (2007–2009). The study shows that production of flowers and pollen grains in a cultivar of the olive tree varies according to the microclimate. Our study also indicates that the olive trees frequently can have up to half a million flowers per tree. Moreover, the total flower production differs between years and study areas. In the Picual cultivar, the average production of pollen grains per anther is usually more than 60 000 grains. The total production of pollen per tree is around 72 000 million on average. The most favourable microclimatic conditions for reproduction in olive trees are found in years and olive growing areas with low temperature and high precipitation records during the months prior to flowering of the olive trees. We hypothesise that olive trees tend to increase their pollen production rate as altitude increases, which can be interpreted as a reproductive strategy to ensure fertilisation.

Keywords: *environmental effect, flower production rate, microclimate, olive, Picual cultivar, pollen production*

Numerous studies on both gymnosperms (e.g. Allison, 1990; Khanduri & Sharma, 2002) and angiosperms (e.g. Tormo Molina et al., 1996; Gómez-Casero et al., 2004; Rovira & Tous, 2005) show that pollen production heavily depends on many different factors such as available resources, soil and environmental conditions and varies considerably among anemophilous species. As pointed out by Rogers (1993), it is not surprising that the amount of pollen produced per anther for the same species varies from year to year. The formation and maturation of pollen grains inside the anther is probably conditioned by genetic (Kaul, 1988) and environmental (García-Mozo et al., 2001) factors. Moreover, pollen production does indeed depend on the meteorological conditions during the pre-flowering period (Fornaciari et al., 1997; Galán et al., 2008), with temperature and precipitation, among other meteorological factors, playing a fundamental role in the process.

Information on the total pollen production of a plant is useful particularly for allergenic plant species (Agnihotri & Singh, 1975) for estimating pollen release into the atmosphere from a specific plant population. This is not only relevant to aerobiology, but also to agriculture. Studies on olive and grape, carried out in different Mediterranean countries, have demonstrated a correlation between the quantity of pollen released during flowering and fruit production (Holm, 1994; Fornaciari et al., 1998; Orlandi et al., 2005). Furthermore, this knowledge can be used to help establish an airborne forecasting system (González-Minero et al., 1998; Galán et al., 2008).

Although the olive tree (*Olea europaea* L.) is an amphiphilous species, its pollination strategy relies mainly on anemophily (Trigo et al., 2008). Olive groves cover a large area of the Iberian Peninsula and are very important not only for agricultural purposes, but also as main cause of pollinosis in the Mediterranean region (d'Amato & Lobefalo, 1989;

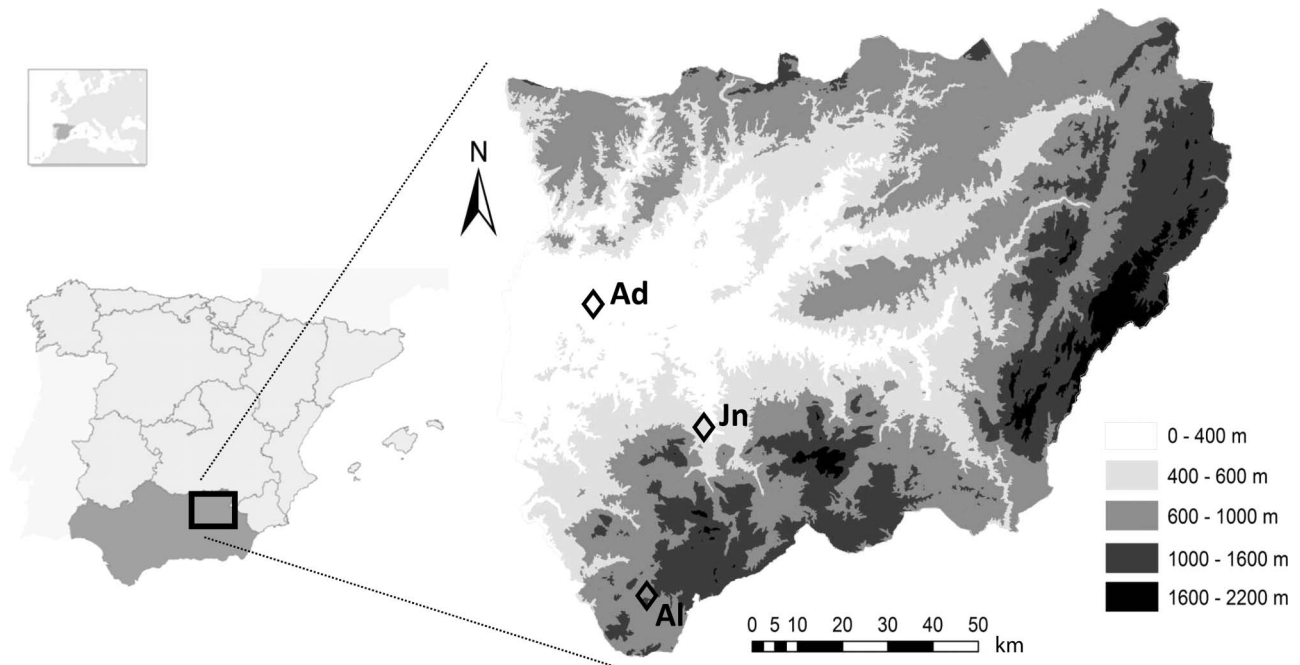


Figure 1. Map of the study localities in Andalusia, Spain. Ad – Andujar, Al – Alcala la Real, Jn – Jaen.

Florido et al., 1999; Díaz de la Guardia et al., 2003; d'Amato et al., 2007). A series of studies have revealed that production and viability of these olive groves depend heavily on, among other things, the cultivar of the olive tree, which sometimes presents cases of male sterility (Rapoport, 2008). In olive and in other hermaphroditic plants, the presence of staminate or imperfect flowers is frequently observed (Reale et al., 2006). According to Cuevas and Polito (2004), the most obvious advantage provided by staminate flower production is the increase of pollen grains available for fertilisation. This is particularly important under anemophily and in species experiencing intense male competition, in which the likelihood of paternity is influenced by the quantity of pollen produced (Stephenson & Bertin, 1983).

This study deals with the Picual cultivar, the most extensive olive cultivar in Spain, located mainly in the provinces of Jaen, Cordoba and Granada (Andalusia, Spain). Picual is a non male-sterile cultivar (Rovira & Tous, 2005), but at present, there is little information on the amount of pollen that this cultivar can produce per anther and flower and, consequently, its average production rate.

The existence of a chronology in the onset of flowering in olive trees have been demonstrated in previous studies (Aguilera & Ruiz Valenzuela, 2009). The length of the flowering period depends on altitude, being delayed and shorter at higher altitudes. This suggests that pollen production could possibly depend on the location of the olive grove with higher

pollen production at higher altitudes compensating for shorter period of flowering.

Therefore, our study aimed to determine the possible impact of altitude and the related microclimatic conditions on the total production of fruiting branches, inflorescences, flowers and pollen of Picual olive trees located in different areas at varying altitudes. For this purpose, we recorded a series of inter-annual differences in meteorological characteristics in order to identify the main weather-related factors affecting pollen production.

Material and methods

Study area

The province of Jaen (13 498 km²) is located in the southeast of the Iberian Peninsula (Figure 1). The climate profile is continental Mediterranean with cold winters and hot and dry summers. Annual average temperature is 15.8 °C and annual average precipitation is 667.4 mm. However, this varies considerably with altitude and topography.

More than 570 000 ha of olive groves cover 41.5% of the total surface area of the province of Jaen. In this intensive monovarietal cultivation, 97% of the olive trees correspond to the Picual cultivar. Since cultivated areas cover a wide altitudinal gradient, the olive groves of Jaen provide an excellent experimental scenario for testing the possible effects of altitude on the total production records of branches, racemes, flowers and pollen. The lowest altitudes are located

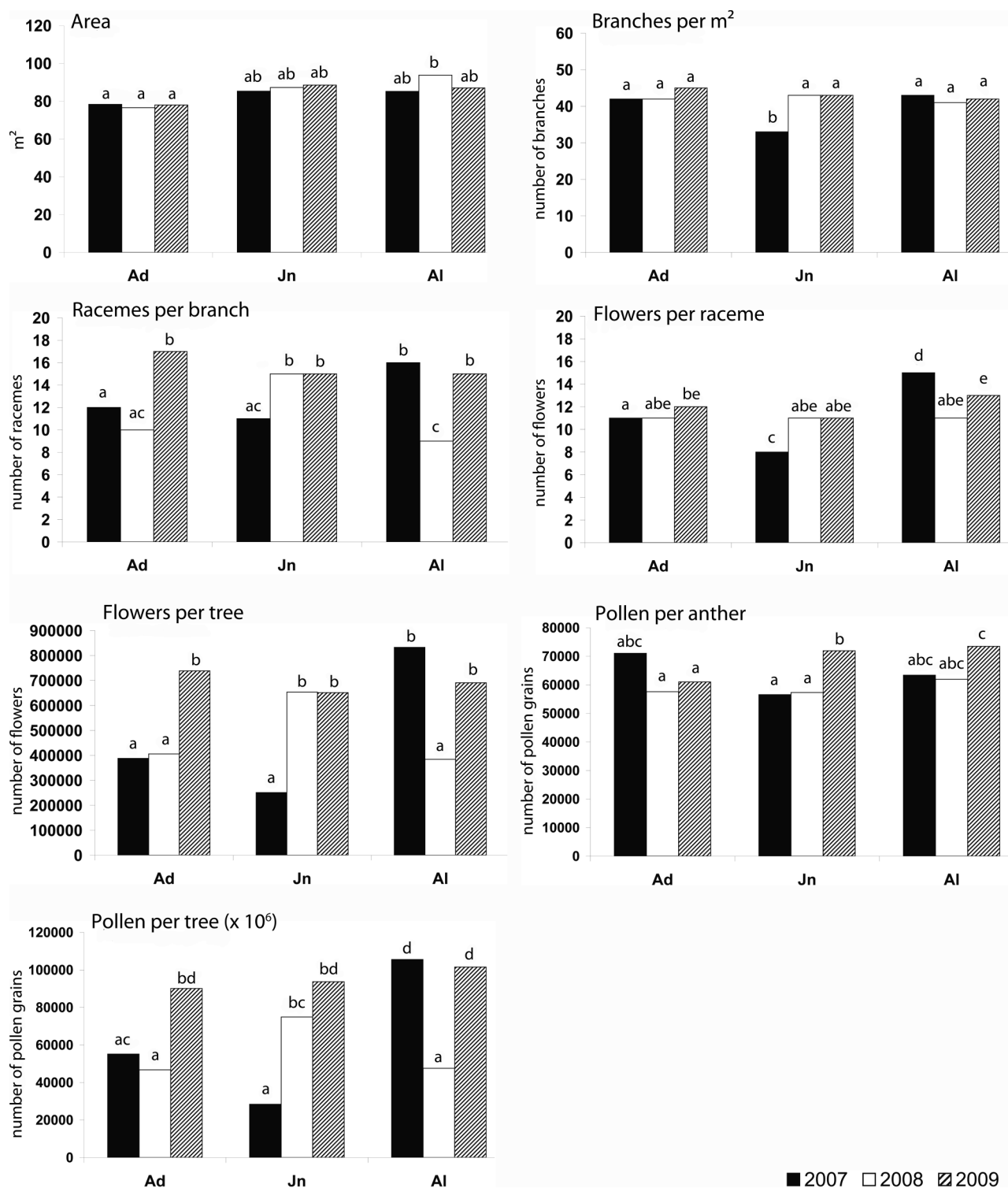


Figure 2. Average values of the fructification area, average branch production per m², racemes per branch, flowers per raceme, flower production per tree and pollen grains per anther and per tree in the olive trees for each study area in the years 2007, 2008 and 2009. Ad – Andujar, Al – Alcala la Real, Jn – Jaen. Different letters indicate statistically different values ($p \leq 0.05$, Tukey test).

within the area of the valley of the Guadalquivir River. By contrast, the highest altitude records tend to be concentrated in the south and southeast of the province, that is, in the Subbetic Cordillera. This

altitudinal gradient imposes variable meteorological conditions on olive groves.

In order to estimate the total production records of flowers and pollen in olive trees and to discover

whether the geographically variable altitude induces differential production rates, we randomly chose a total of 90 Picual cultivar trees distributed in olive groves all over the altitudinal zonation: Andujar (Ad, 248 m above sea level [a.s.l.]), Jaen (Jn, 590 m a.s.l.) and Alcala la Real (Al, 942 m a.s.l.) (see Figure 1). Records were taken from 30 individual trees per study site. The study was conducted during the olive tree flowering period in the years 2007, 2008 and 2009.

Estimation of total pollen production

The average pollen production rate was estimated by recording a series of parameters in two successive stages: (1) estimation of the number of flowers per tree; (2) estimation of number of pollen grains per flower.

In order to estimate the number of flowers per tree, it was necessary to record certain data per individual

tree first: The number of flowers per raceme (or inflorescence/panicle) was reckoned by counting the flowers on the racemes of two branches, one each in northern and southern exposure, with the subsequent calculation of average values. The estimation of the number of racemes per branch (or fruiting branch) was similar to the previous procedure, but the counting involved the racemes on both branches. Finally, the number of branches per m² of the tree crown was estimated by counting the branches in randomly located grids of 1 m² in cardinal points of each tree crown with the subsequent calculation of average values. Finally, the tree crown area (m²) was calculated by means of the formula suggested by Pastor Muñoz-Cobo et al. (2001): $S = (3\pi/4) \times D^2$, where S is the surface (in m²) and D the mean tree crown diameter (in m). In order to estimate the total production of flowers per tree, the recorded data were extrapolated to the total tree crown area.

Table I. Summary of the main average data for the years 2007, 2008 and 2009.

	Andujar	Jaen	Alcala la Real	Mean
<i>2007</i>				
Maximum D (m)	6.52 (± 0.97)	6.72 (± 0.74)	6.58 (± 0.55)	6.60 (± 0.85)
Minimum D (m)	4.90 (± 0.82)	5.24 (± 0.89)	5.42 (± 0.63)	5.18 (± 0.78)
Average D (m)	5.71 (± 0.77)	5.98 (± 0.65)	6.00 (± 0.37)	5.89 (± 0.61)
Area (m ²)	78.40 (± 21.19)	85.39 (± 19.27)	85.28 (± 10.53)	83 (± 17.71)
Branches/m ²	42 (± 11.38)	33 (± 5.16)	43 (± 4.93)	39 (± 8.88)
Racemes/branch	12 (± 3.76)	11 (± 1.92)	16 (± 2.79)	13 (± 3.54)
Flowers/raceme	11 (± 1.89)	8 (± 1.80)	15 (± 2.26)	11 (± 3.51)
Flowers/tree	387 957 ($\pm 190 388$)	251 080 ($\pm 132 815$)	832 564 ($\pm 196 602$)	499 440 ($\pm 308 776$)
Pollen/anther	71 065 ($\pm 18 192$)	56 587 ($\pm 14 290$)	63 403 ($\pm 14 822$)	63 685 ($\pm 16 693$)
Pollen/flower	142 131 ($\pm 36 384$)	113 175 ($\pm 28 579$)	126 807 ($\pm 29 644$)	127 371 ($\pm 33 386$)
Pollen/tree ($\times 10^6$)	55 140 ($\pm 26 513$)	28 416 ($\pm 15 031$)	105 574 ($\pm 24 930$)	63 926 ($\pm 39 948$)
<i>2008</i>				
Maximum D (m)	6.57 (± 0.75)	6.60 (± 1.03)	7.08 (± 0.66)	6.75 (± 0.82)
Minimum D (m)	5.08 (± 0.99)	5.48 (± 0.67)	5.51 (± 0.66)	5.36 (± 0.78)
Average D (m)	5.82 (± 0.99)	6.04 (± 0.75)	6.29 (± 0.44)	6.06 (± 0.68)
Area (m ²)	76.65 (± 21.28)	87.34 (± 21.30)	93.87 (± 13.60)	85.95 (± 18.72)
Branches/m ²	42 (± 9.62)	43 (± 7.38)	41 (± 7.48)	42 (± 8.18)
Racemes/branch	10 (± 2.81)	15 (± 3.37)	9 (± 1.78)	11 (± 2.65)
Flowers/raceme	11 (± 2.36)	11 (± 2.47)	11 (± 1.40)	11 (± 2.08)
Flowers/tree	405 894 ($\pm 250 404$)	653 438 ($\pm 305 068$)	384 096 ($\pm 166 264$)	481 143 ($\pm 240 579$)
Pollen/anther	57 595 ($\pm 12 601$)	57 320 ($\pm 16 936$)	61 930 ($\pm 11 231$)	58 948 ($\pm 13 590$)
Pollen/flower	115 190 ($\pm 25 203$)	114 640 ($\pm 33 872$)	123 860 ($\pm 22 463$)	117 897 ($\pm 5 956$)
Pollen/tree ($\times 10^6$)	46 750 ($\pm 28 844$)	74 910 ($\pm 34 973$)	47 574 ($\pm 20 593$)	56 413 ($\pm 16 024$)
<i>2009</i>				
Maximum D (m)	6.47 (± 0.64)	6.68 (± 1.09)	6.93 (± 0.58)	6.70 (± 0.77)
Minimum D (m)	4.97 (± 0.77)	5.48 (± 0.67)	5.19 (± 0.69)	5.22 (± 0.72)
Average D (m)	5.72 (± 0.63)	6.083 (± 0.78)	6.06 (± 0.46)	5.95 (± 0.62)
Area (m ²)	78.09 (± 17.07)	88.61 (± 22.62)	87.10 (± 13.31)	84.60 (± 17.66)
Branches/m ²	45 (± 8.69)	43 (± 7.20)	42 (± 6.51)	43 (± 7.47)
Racemes/branch	17 (± 3.34)	15 (± 3.73)	15 (± 2.97)	15 (± 3.35)
Flowers/raceme	12 (± 1.84)	11 (± 1.14)	13 (± 1.70)	12 (± 1.56)
Flowers/tree	738 459 ($\pm 319 046$)	651 368 ($\pm 308 773$)	691 001 ($\pm 232 763$)	693 609 ($\pm 86 861$)
Pollen/anther	61 020 ($\pm 16 781$)	71 942 ($\pm 14 029$)	73 490 ($\pm 15 571$)	68 817 ($\pm 15 460$)
Pollen/flower	122 040 ($\pm 33 562$)	143 885 ($\pm 28 058$)	146 980 ($\pm 31 142$)	137 635 ($\pm 2 759$)
Pollen/tree ($\times 10^6$)	90 121 ($\pm 38 936$)	93 722 ($\pm 44 428$)	101 563 ($\pm 34 212$)	95 136 ($\pm 5 850$)

Note: D , tree crown diameter.

The estimation of the number of pollen grains per flower required the previous estimation of pollen production per anther. Five flowers in their pre-flowering stage (bud stage) of two randomly chosen trees were selected in each study area for the years 2007 and 2008. For the year 2009, we increased the number to four individual trees. The method suggested by Dafni (1992) was applied. One anther of each flower was put in an Eppendorf tube containing 300 μl of a distilled water solution with 70% ethanol and methylene blue (0.5%). The anther was macerated and stirred to obtain a homogeneous product. The subsequent counting of pollen grains was done with a light microscope by taking 20 μl aliquots placed on microscopic slides. Three aliquot samplings were processed from each tube. The estimation of the number of produced pollen grains per flower was made by extrapolation of the recorded data to the total sampling volume and the subsequent multiplication by the number of anthers per flower (two in the case of olive trees).

Statistical analysis

Variance analysis (one-way ANOVA) was carried out to detect whether differences in flower and pollen production were induced by the geographically variable environmental conditions. This comparative analysis between study areas was implemented both in annual and global terms. For the latter approach, we used the data of the three-year study as a whole. A factorial ANOVA including, at the same time, location and year, was used to reveal if both factors and their interaction have a significant influence on the measured parameters.

For the study of the impact of meteorological variables on the flowers and pollen production rate of olive trees, we implemented parametric and non-parametric correlation analyses in relation to data normality. We used 95% as confidence levels.

The meteorological variables considered in our study were arranged in two groups, the monthly average and accumulated values, respectively, for pre-flowering months. The variables included in the first group were maximum temperature (T_{max} , in $^{\circ}\text{C}$), minimum temperature (T_{min} , in $^{\circ}\text{C}$), relative humidity (RH, in %), solar radiation (Rad, in MJ/m^2) and precipitation (P_p , in mm); in the second group, accumulated mean temperature (T_{acme} , in $^{\circ}\text{C}$), accumulated solar radiation (Rad_{ac} , in MJ/m^2) and accumulated precipitation (P_{pac} , in mm) (Díaz de La Guardia et al., 2003; García-Mozo et al., 2008).

The meteorological data were provided by the Agroclimatic Station Network of the Andalusian Consejería de Agricultura y Pesca, Sevilla. The

information provided was recorded from the weather stations nearest to the study areas.

Prior to the correlation analysis, we carried out a principal component analysis in order to reduce the final number of variables involved and to obtain as much useful information as possible. The criterion for the selection of the number of factors or components was that they should accumulate about 80% of the explained variance. For all statistical analysis, STATISTICA 7.0 software was used.

Results

Fructification area, branch production and racemes

Average fructification area. — The fructification area does not vary significantly from one year to the next (Ad: $F = 0.039$, $p = 0.962$; Jn: $F = 0.166$, $p = 0.847$; Al: $F = 3.894$, $p = 0.054$; for all $p \leq 0.05$). In the study area, olive trees have an average fructification area of 84.51 m^2 (± 18.03). The olive trees of Andujar generally show lower values than those of Jaen and Alcala la Real (Figure 2; Table I). In Andujar, the tree crown area varied between 76.65 m^2 (± 21.28) and 78.40 m^2 (± 21.19), whereas in Jaen and Alcala la Real, these values were between 85.28 m^2 (± 10.53) and 93.87 m^2 (± 13.60). Nevertheless, significant differences between study areas were only found for the year 2008 ($F = 6.142$, $p = 0.003$) between olive trees of Andujar and Alcala la Real.

Average branches production per m^2 . — The recorded average values were 39 (± 8.88) branches for the year 2007, 42 (± 8.18) for 2008 and 43 (± 7.47) for 2009. Significant differences between study areas were only found in 2007 ($F = 13.917$, $p = 0.000$). In that year, trees at Jaen showed the lowest branch production values with an average branch record of 33 (± 5.16) per m^2 , followed by the trees at Andujar with 42 (± 11.38) and Alcala la Real with 43 (± 4.93) branches per m^2 (Figure 2). When the records of average branch production per m^2 were considered altogether, no significant differences between study areas were found ($F = 2.716$, $p = 0.067$; Figure 3).

Average raceme production per branch. — Significant differences between years and areas were recorded for this parameter. Moreover, significant interactions between study area and year were found ($F = 29.059$, $p = 0.000$). In 2007, the trees in Alcala la Real exhibited the highest raceme production with an average value of 16 (± 2.79) racemes per branch. Andujar and Jaen trees showed considerably lower values, with an average production record of 12 (± 3.76) and 11

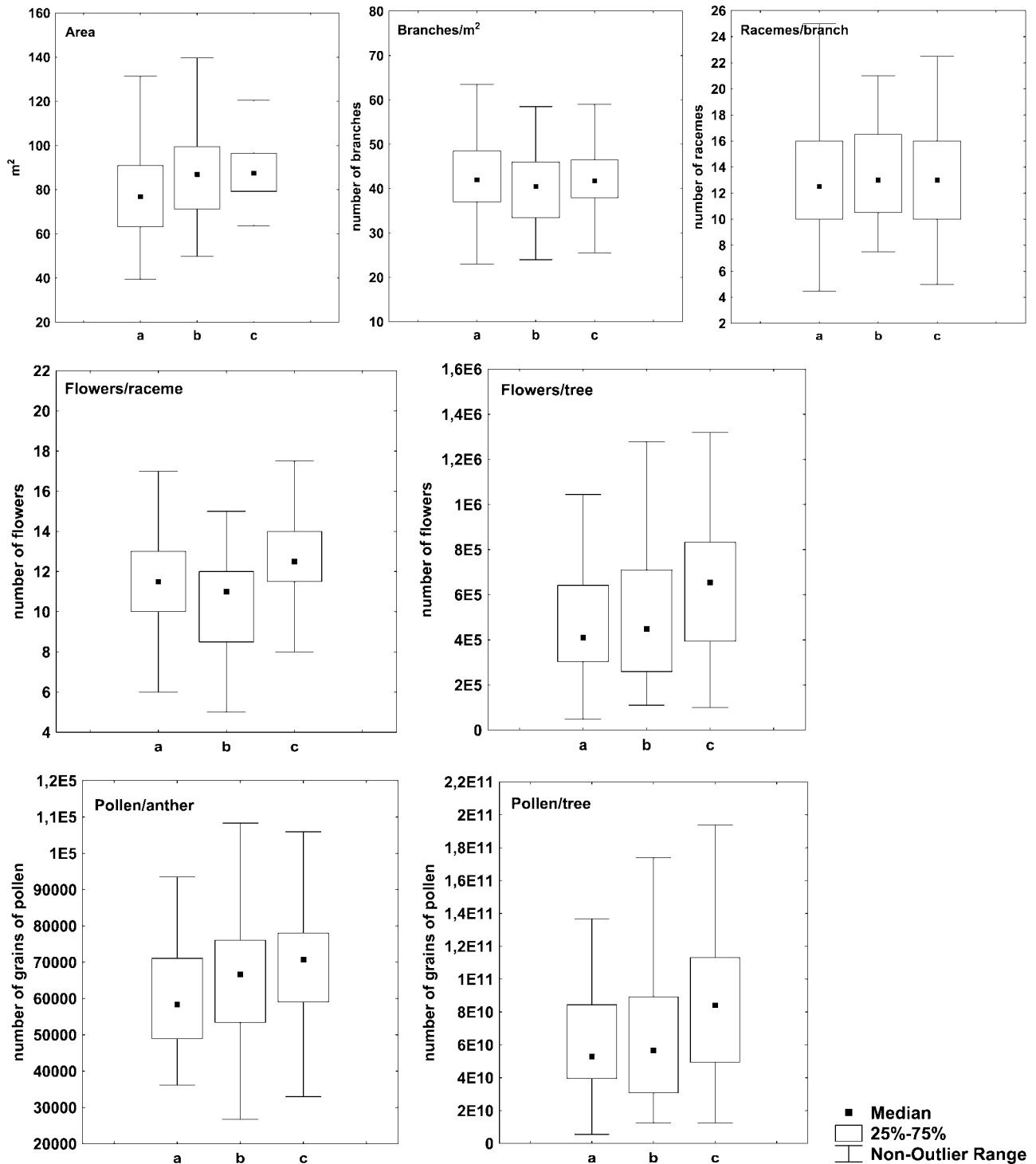


Figure 3. Average values of the variables under study for the three-year period (2007, 2008 and 2009 considered in total); a – Andujar, b – Jaen, c – Alcala la Real.

(± 1.92) racemes per branch, respectively (Figure 2). In 2008, trees at Jaen had the highest raceme production per branch with an average value of 15 (± 3.37), whereas trees at Andujar and Alcala la Real had lower records with 10 (± 2.81) and 9 (± 1.78),

respectively. In 2009, Andujar was the area with the highest raceme production rate with an average record of 17 (± 3.34) racemes per branch, while trees at Jaen and Alcala la Real had similar production rates with an average of 15 (± 2.97 – 3.73) racemes

per branch. Despite recording significant annual differences between the study areas, no significant differences in raceme production per branch were found when the data of all three years were considered together ($F = 0.816$, $p = 0.443$; Figure 3).

Flower production

Average flower production per raceme. — This parameter showed significant inter-annual variation in the three olive growing areas (Ad: $F = 6.317$, $p = 0.002$; Jn: $F = 33.808$, $p = 0.000$; Al: $F = 23.795$, $p = 0.000$; for all $p \leq 0.05$). The average flower production per raceme was significant different for the study areas only in 2007 ($F = 81.410$, $p = 0.000$), when the record ranged from 11 (± 1.92) to 16 (± 2.79). The olive trees of Alcala la Real showed the highest flower production rates. In 2008, the average value for all study areas was 11 (± 2.08) flowers per raceme. In 2009, the production record ranged from 11 (± 1.14) to 13 (± 1.70) flowers per raceme, with the area of Alcala la Real once again showing the highest production rate, although without significant differences. On a global scale, differences were found ($F = 31.639$, $p = 0.000$), where the olive groves of Alcala la Real had the highest flower production rates per raceme. Here, the contribution of the records registered in 2007 and 2009 were very important for the study area.

Average flower production per tree. — This parameter was different between olive growing areas ($F = 4.433$, $p = 0.012$) and differed significantly between the three years within each area (Ad: $F = 16.978$, $p = 0.000$; Jn: $F = 21.815$, $p = 0.000$; Al: $F = 39.264$, $p = 0.001$; for all $p \leq 0.05$). In 2007, the olive trees had a total average production of 499 440 ($\pm 308 776$) flowers. With about 833 000 flowers per tree, the olive trees of Alcala la Real had a production rate that was clearly higher than in the other study areas. In 2008, the total average production was 481 143 ($\pm 240 579$) flowers per tree. The highest production rate corresponded here to the olive trees of Jaen with an average value of 653 438 ($\pm 305 068$) flowers per tree. In 2009, the total average production was 693 609 ($\pm 86 861$) flowers per tree with no significant differences between the study areas, although in Andujar and Alcala la Real, records were considerably higher than in the previous year (Figure 2). When data are considered in total throughout the three-year period, we observed that flower production per tree was highest in Alcala la Real with an average record of 635 887 ($\pm 198 543$) flowers, followed by Jaen and Andujar with a similar value of about 520 000 flowers per tree (Figure 3).

Table II. Correlation coefficients between meteorological variables and branch production per m², racemes per branch, flowers per raceme and pollen grains per anther in olive trees.

	Branches/ m ²	Racemes/ branch	Flowers/ raceme	Pollen/ anther
Branches/m ²	1.00	0.58	0.53	0.35
Racemes/branch	0.58	1.00	0.13	0.34
Flowers/raceme	0.53	0.13	1.00	0.36
Pollen/anther	0.35	0.34	0.36	1.00
T_{\max} (January)	-0.28	-0.37	-0.45	-0.83*
RH (January)	0.02	0.05	0.68*	0.04
P_{pac} (January)	0.21	0.19	0.68*	0.63
T_{\min} (February)	-0.61	-0.67*	-0.63	-0.58
P_{pac} (March)	0.15	0.37	0.47	0.89*
T_{\min} (April)	-0.52	-0.59	-0.40	-0.67*
RH (April)	-0.38	0.07	-0.70*	-0.24

Note: T_{\max} , mean maximum temperature; T_{\min} , mean minimum temperature; RH, mean relative humidity; P_{pac} , accumulated precipitation; * $p < 0.05$.

Pollen production

Average production of pollen grains per anther. — With average records of 63 685 ($\pm 16 693$) and 58 948 ($\pm 13 590$) pollen grains per anther, respectively, the study areas showed similar figures in 2007 and 2008. However, in 2009, significant differences appeared between the three olive growing areas (Figure 2): The average production of pollen grains per anther ranged from 61 020 ($\pm 16 781$) grains in trees at Andujar to 73 490 ($\pm 15 571$) in Alcala la Real. However, significant differences between years were only found in olive trees of Jaen. That area showed the highest production of pollen grains per anther in 2009 with respect to the previous years. Significant differences in pollen production per anther were found when the data of all three years were considered together ($F = 6.479$, $p = 0.001$; Figure 3).

Total pollen production per tree. — Significant differences between years and areas were detected. Significant interaction between study area and year were also found ($F = 21.290$, $p = 0.000$). Results reveal a variable production rate depending on both year and study area. The year 2009 generally showed the highest pollen production records per tree with an average value of $95\,136 \times 10^6$ ($\pm 5850 \times 10^6$) (Table I). By contrast, pollen production record per tree was lowest in 2008, with an average value of $56\,413 \times 10^6$ ($\pm 16\,024$) pollen grains. With $63\,926 \times 10^6$ ($\pm 39\,948$) pollen grains per tree, 2007 was in between the two other years. In the years 2007 and 2008, significant differences between study areas were detected. Trees at Alcala la Real showed very much higher pollen production in 2007 ($105\,574 \times 10^6 \pm 24\,930 \times 10^6$) as did Jaen in 2008 ($74\,910 \times 10^6 \pm 34\,973 \times 10^6$; Figure 2). In 2009,

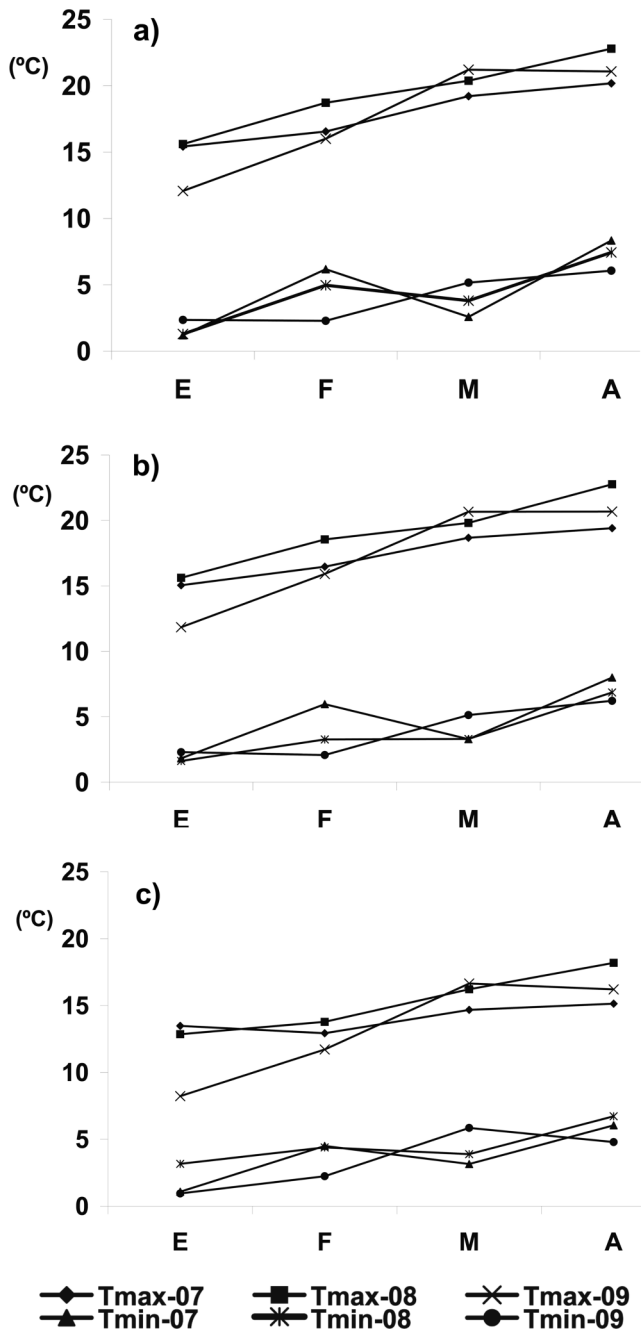


Figure 4. Mean maximum and minimum temperatures for the pre-flowering months of 2007, 2008 and 2009 (figure codes have the same meaning as in Table II). a – Andujar, b – Jaen, c – Alcala la Real; E – January, F – February, M – March, A – April.

no differences between study areas were observed, although trees at Alcala la Real again had a production record of more than $100\,000 \times 10^6$ pollen grains. When all pollen production records per tree were considered together, significant differences between the study areas were found ($F = 7.328$, $p = 0.000$; Figure 3). In general, the lowest average values were recorded in Andujar and Jaen.

Correlation analysis

Variables showing significant correlation included T_{\max} , RH and P_{pac} in January, T_{\min} in February, P_{pac} in March and T_{\min} and RH in April (Table II). Branch production per m^2 does not seem to depend on meteorological variables; no significant correlation value was recorded. Average raceme production per branch correlated negatively to T_{\min} in February. Relative humidity and P_{pac} in January correlated significantly and positively to the average flower production per raceme. Meanwhile, RH had a negative effect in April. Pollen production per anther is negatively affected by T_{\max} in January and T_{\min} in April. Accumulated precipitation in March had a positive effect.

The temperature pattern was similar for the pre-flowering periods of 2007 and 2008 with maximum temperatures between 15°C and 23°C in Andujar and Jaen and between 12°C and 19°C in Alcala la Real (Figure 4). Minimum temperatures were between 2°C and 8°C in Andujar and Jaen and between 1°C and 7°C in Alcala la Real. In general, 2009 had lower temperature records than the two previous years.

Humidity percentages in January were generally similar for the three study years with values around 80% (Figure 5A). However, in April 2008 and 2009, humidity percentages were lower (around 60% for 2008 and 70% for 2009).

The total precipitation for January had higher records than for March in all three years, and 2009 was the year with by far the highest precipitation records for both months (Figure 5B). In general, Alcala la Real exhibited the highest precipitation records, particularly for January.

Discussion

This study revealed that in the same olive grove, the fructification area of the olive trees does not vary inter-annually, also not from one olive growing area to another. No differences in flower/branch production per m^2 were observed between the three olive growing areas, despite the biomass reduction between years, as a result of the regular pruning of the trees usually occurring every two years. Fruit/branch production was not shown to be dependent on meteorological conditions, which seems to indicate that it is rather related to inherent features of the cultivar concerned. According to some authors, the growth of branches is closely associated with the number of fruits on the tree (Fernandez-Escobar et al., 1992; Barranco et al., 2008). In bearing years, the distribution of assimilated resources is mostly directed to the developing

fruits with correspondingly reduced vegetative tree growth, reduced growth of branches and fewer potential flowering points during that year. For this reason, every bearing year is followed by a non-bearing year, a common phenomenon in olive trees and other fruit trees, which is known as *vecería* or alternate bearing (Fernandez-Escobar et al., 1992; Barranco et al., 2008).

We did not observe *vecería* in branch formation, but raceme production per branch behaved according to a clear biennial pattern, and the year with high raceme production was followed by a year of comparatively low production. Two main reasons can probably explain this, as there are the inhibition of floral induction as a result of fruit presence on the tree and the probable action of gibberellins synthesised inside the seeds (Stutte & Martin, 1986; Fernandez-Escobar et al., 1992), and, as has been revealed in this research, the effect of meteorological variables on the formation of racemes on the tree, in particular, the average minimum temperature in February, the month when the swelling of the meristematic bulge and floral differentiation usually take place in most olive tree varieties (Mehri & Kamoun-Mehri, 1995; Barranco et al., 2008).

The year 2009 had the highest raceme production per branch, probably because minimum temperatures were significantly lower than in the previous years. When precipitation is abundant under these conditions, reinforced vegetative growth seems to encourage the production of floral buds (Barranco et al., 2008). It is important to note that, despite the biennial differences observed in the number of racemes per branch within the same olive growing area, there are no differences when all areas are considered together. This alternate behaviour occurs independently in each olive growing area and, consequently, there is no synchronised *vecería* throughout the olive groves of the province of Jaen.

The average flower production per raceme also varied between years and study areas. Recent studies have shown that the amount of flowers on the inflorescence is closely related to both the bearing state of the tree and the harvesting period, with early harvesting of fruits in November having a positive impact on the formation of floral buds (Barranco et al., 2008; Castillo-Llanque et al., 2009). This feature of olive trees is heavily conditioned by meteorological conditions during the months prior to flowering. Both relative humidity and accumulated

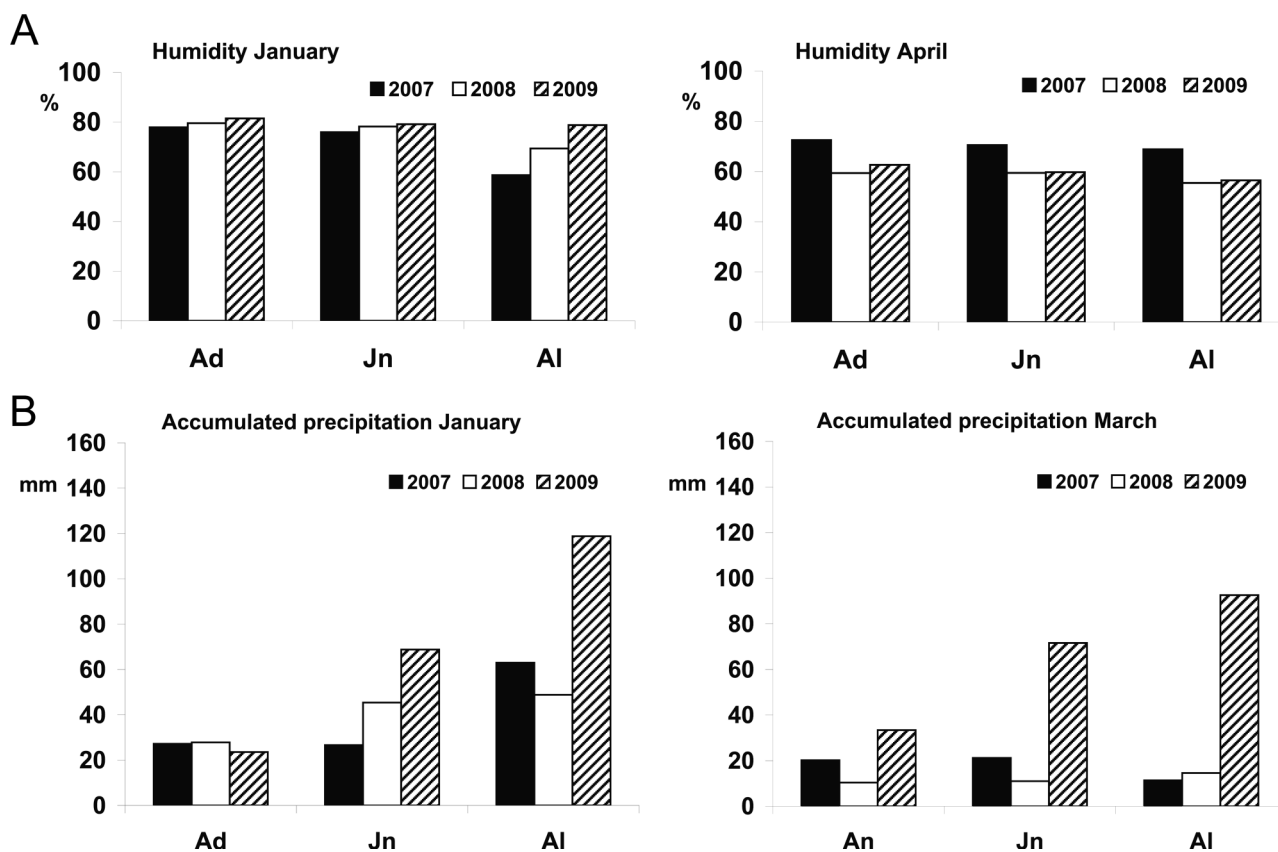


Figure 5. Mean relative humidity for January and April (A) and accumulated precipitation for January and March (B) in 2007, 2008 and 2009 for the three study areas. Ad – Andujar, Jn – Jaen, Al – Alcala la Real.

precipitation during January had a clearly positive impact on flower production. By contrast, humidity in April, the month before the onset of flowering, seemed to have a negative impact on this feature of olive trees. Generally speaking, the trees in the area of Alcalá la Real, located 900 m a.s.l., produced larger amounts of flowers per raceme than the trees at Andujar and Jaén. With the typical lower temperature pattern and higher precipitation, typical of areas located at these altitudes, the area has a privileged position in this respect.

Olive trees could have up to half a million flowers per tree. In our field research, we recorded values significantly higher than the average of 314 000 flowers per tree recorded by Tormo Molina et al. (1996). As a result mainly of the differential raceme production and the tree crown area, the total flower production of olive trees differed between study areas. Once again, it is the olive trees growing at higher altitudes, which have higher flower production rates. These areas exceed the total average flower production of olive groves located in areas below 600 m a.s.l. by 18%.

In the Picual cultivar, the average production of pollen grains per anther is usually more than 60 000 grains with records even higher than 73 000 grains during climatically favourable years. Our data differ from these records provided by Tormo Molina et al. (1996) for this species. These authors recorded between 83 000 and 104 000 pollen grains per anther. This is probably due to the particular cultivar they studied. Other species of the Oleaceae family, such as *Fraxinus excelsior* L. or *F. angustifolia* Vahl., also show variable quantities of pollen production per anther with 12 500 grains for the former (Pohl, 1937) and 18 000–87 000 for the latter (Tormo Molina et al., 1996). In line with the data provided by Fornaciari et al. (1997), Gómez-Casero et al. (2004) or Galán et al. (2008), our records confirm that temperature and precipitation are the variables, which mostly affect the pollen production of olive trees. According to our data, high values of maximum and minimum temperatures recorded during the pre-flowering months correlate inversely to the average production of pollen per anther. By contrast, precipitation in early spring proves to have a positive effect on pollen production. This is further supported by the dramatically larger pollen production in 2009, when the pre-flowering period took place at temperatures lower than in previous years and not only were precipitation records higher, but also meteorological conditions (e.g. temperature) were more favourable. If these favourable conditions are not met, pollen production may be reduced. The same is true for the total production of pollen per tree. In 2009, the total production of

pollen exceeded 95 000 million on average, as compared to the average 56 413 million recorded in 2008, a year climatically less favourable. This could explain why olive trees located at higher altitudes frequently produce over 100 000 million pollen grains per tree, the highest records of all. For this species, Tormo Molina et al. (1996) recorded highly variable values, ranging from 29 000 to 108 000 million pollen grains per tree.

Some authors maintain that anemophilous tree species produce a constant amount of pollen by trade-off with some of their sexual and pollination strategies. This behaviour has already been recorded in anemophilous species belonging to the genera *Quercus* or *Salix* (Tormo Molina et al., 1996; Gómez-Casero et al., 2004). However, in the case of olive trees, we did not record any trade-off between pollen production rate and different sexual characters such as average branch, raceme or flower production.

Our research shows that the Picual cultivar of olive behaves differently according to the microclimate, with differential production of flowers and pollen grains. The most favourable microclimatic conditions for both flower and pollen production rates in olive trees are found in years or/and olive growing areas with low temperature and high precipitation records during the pre-flowering months of the trees. These conditions promote the formation of flowers and contribute positively to an increase in pollen production.

Therefore, the altitude-induced microclimatic conditions determine the physiological response of olive trees. Olive reproductive cycle is characterised by bud formation during the previous summer, dormancy during the cold period, budburst in late winter and flower structure development from budburst to flowering in spring. Of all the factors affecting plant development, temperature and precipitation exert the strongest effect on vegetation development and especially on flowering (Cenci et al., 1997; Aguilera & Ruiz Valenzuela, 2009). The flowering induction of the buds is a noteworthy phase, because it can express a great part of future flower production variability, taking into consideration the fact that it is directly involved in the reproductive process (Rallo & Martín, 1991). The exact time of induction in olive is unknown. However, several studies indicate that environmental factors during winter, especially the temperature, play a role in flower induction and subsequent initiation (Hackett & Hartmann, 1967; Badr & Hartmann, 1971; Hartmann & Whisler, 1975; Fernandez-Escobar et al., 1992). This process is positively influenced by low temperatures and the higher rainfall, meteorological factors, which depend on the particular situation of each olive growing area (Nair Taheen et al., 1995; results of this study).

We also hypothesise that olive trees tend to increase their pollen production rate as altitude increases. The variable, location-dependent pollen production rate recorded for the Picual cultivar clearly supports this conclusion. The olive groves thriving at higher altitudes typically with lower temperatures and higher precipitation produce in general the highest amounts of pollen. This can be interpreted as a reproductive strategy to ensure fertilisation, since greater emission of pollen could compensate for the shorter flowering period of the olive groves growing at higher altitudes (Aguilera & Ruiz Valenzuela, 2009).

Several authors have confirmed that olive harvest production records are highly correlated to pollen availability, emission, receptivity and, consequently, to the total number of flowers (González-Minero et al., 1998; Fornaciari et al., 2002, 2005; Galán et al., 2008). The results of this study contribute to the understanding of the microclimatic effect on different parameters, such as the average inflorescence and flower production rate or the amount of pollen in the olive tree.

It would be interesting to widen our study to relate total flower and pollen production with the availability of water and nutrients, and also with soil properties, factors that could greatly influence reproductive characteristics of a plant species, and, therefore, should be taken into account for future studies.

Conclusion

The physiological response of olive trees behaves differently according to the microclimate, with differential average production of flowers and pollen grains. Olive trees tend to increase their flower and pollen production rate as altitude increases. Low temperature and high precipitation during the months previous to the flowering period are the meteorological variables that affect the flower and pollen production of olive trees most.

Acknowledgements

The authors are grateful to the University of Jaen for a pre-doctoral grant and to the Agroclimatic Station Network of the Andalusian Consejería de Agricultura y Pesca, Sevilla, Spain, for meteorological data.

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