

Time trend in the viability of pollen grains in the ‘Picual’ olive (*Olea europaea* L.) cultivar

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The aim of this study was to evaluate the viability and longevity of the pollen grains of the olive cultivar ‘Picual’, the most extensive and important olive cultivar in the Iberian Peninsula. Over a 3-year period (2007–2009), viability of the pollen grains of 15 ‘Picual’ cultivar trees was measured using the Fluorochromatic Reaction (FCR). These olive trees were distributed along different altitudinal areas of the province of Jaen (southeast Iberian Peninsula). The percentages of viability did not show any geographical variability. In general, the percentage of viability did not vary from one year to another. With an average value of 49%, the viability of the pollen in the cultivar ‘Picual’ could be classified as medium-range. ‘Picual’ pollen grains have the highest viability after 24 hours of anther dehiscence. Viability declines rapidly with time. The longevity of the pollen grains was about seven days. The first three days after anthesis are the most important for fertilisation. A rapid decline in pollen viability may considerably reduce effective fertilisation and could negatively influence fruit production. For this reason, these findings should be considered for the improvement of olive fruit yields.

Keywords: fluorescein diacetate; *Olea europaea* L.; pollen viability; trend line

1. Introduction

Olive trees (*Olea europaea* L.) are one of the most extensively cultivated fruit crops in the world, playing a social and economic role of extreme importance (Barranco et al. 2008). These evergreen trees or shrubs are native to the Mediterranean, Asia and Africa. Tree height ranges from 8 to 15 m. The trees have silvery green leaves and are famous for their gnarled and twisted trunks.

Olive trees have abundant small, fragrant, cream-coloured flowers. There are two kinds of olive flowers: the perfect flower, which contains both male and female reproductive organs, and the staminate flower, which contains only stamens. In olive trees and other hermaphrodite 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 in the number of pollen grains available to achieve fertilisation. This is particularly important under anemophily and, as pointed out by Stephenson and Bertin (1983), in species experiencing intense male competition in which the likelihood of paternity is influenced by the quantity of pollen produced. Although the olive tree is an amphiphilous species, its pollination strategy relies mainly on anemophily, with most olive cultivars being

self-fertile (Trigo et al. 2008). A series of studies have revealed that the pollen production and viability of olive groves depend heavily on (among other things) the cultivar of the olive tree, which sometimes presents cases of male sterility (Rapoport 2008).

The fruit is a small drupe which is 1–1.25 cm long and is harvested during its green to purple stage for canned olives and olive oil production. The fruit’s pericarp is usually 60–70% oil, and typical yields are 1.5–2.2 kg of oil per tree per year. Green olives are harvested from the end of September to the middle of November, blond olives from the middle of October to the end of November and black olives from the middle of November to the end of January or early February (Rallo et al. 2005).

With 39% of the world’s total olive oil output, Spain is the biggest olive-growing area in the world. The province of Jaen is responsible for 25% of the total Spanish olive production (Barranco et al. 2008). Olive groves cover 41.5% (571,000 ha) of the total surface area of this province. In this intensive monovarietal cultivation, 97% of olive trees belong to the ‘Picual’ cultivar, which comprises about 50% of Spain’s olive production and about 20% of world olive production. The fruit of this cultivar has a strong but sweet flavour and its oil has some of the best chemical properties found in olive oil (Barranco et al. 2008).

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As for other fruit tree species, olive trees are characterised by plentiful flowering followed by poor fruit set and, consequently, low yields (Stephenson 1981). Cuevas et al. (2009) found that the cultivar 'Picual' was highly self-incompatible with a fruit set of 2%. The maximum fruit set they could obtain was 40% and it was achieved from pollination carried out 4 days after anthesis. This problematically low fruit set is partly due to a wide range of issues concerning self-incompatibility. The spectrum of causes varies from self-compatibility to total male sterility among the cultivars (Moutier 2002). Furthermore, different olive cultivars have a low fecundation capability due to the reduced viability and germinability of their pollen grains (Bini 1984; Rovira & Tous 2005; Ferri et al. 2008).

Although pollen viability has already been studied in numerous Spanish and Mediterranean olive cultivars (Cuevas et al. 2001; Rovira & Tous 2005; Reale et al. 2006; Ferri et al. 2008), there have been few studies on the olive cultivar 'Picual'. Furthermore, a study on the longevity of the viability of 'Picual' pollen grains has not yet been conducted. The loss of viability, which is a continuous but arbitrary process, implies the need to determine the period of time when pollen grains are potentially viable.

There are a number of contexts where it is important to test the functional quality of pollen: for example, in order to assess the fertility of parent plants, to improve crops or to monitor the vigour of pollen during storage (Heslop-Harrison et al. 1984). Although pollen viability is essential for fertilisation and consequently for the olive fruit set, there are other factors which can limit the reproductive success in this species such as pollen longevity, ovule production, stigma receptivity, pistil abortion, pollen import and incompatibility systems (Cuevas et al. 2001, 2009).

The aim of this study was to evaluate the viability and longevity of the pollen grains of the olive cultivar 'Picual' by means of the Fluorochromatic Reaction (FCR).

2. Materials and methods

2.1. Study area

The province of Jaen (13,498 km²) is located in the southeast of the Iberian Peninsula (Figure 1). The climate is Mediterranean continental with cold winters and hot, dry summers. Annual average temperature is 15.82°C and annual average rainfall is 667.4 mm. However, this varies considerably depending on the altitude and topography of the province. The cultivated area covers a wide altitudinal gradient, which induces variable meteorological conditions in olive groves. The present study was carried out in three

different altitudinal olive-growing areas in the province. Andujar (Ad; 248 m a.s.l.) is located in the area of the Guadalquivir River with the lowest altitudes in the province. The annual average temperature is 18.1°C, and annual average rainfall is 463.9 mm. Jaen (Jn; 590 m a.s.l.) is located at a medium-high altitude in the province. The annual average temperature is 16.9°C and annual average rainfall is 578.3 mm. Alcala la Real (Al; 942 m a.s.l.) is located in the Subbetic Cordillera, which has the highest altitudes. The annual average temperature is 14°C and annual average rainfall is 681 mm (Valle et al. 2004).

2.2. Pollen viability

The viability of the pollen grains of 15 'Picual' cultivar trees (5 individuals from each study site) was measured over a 3-year period (2007–2009) (Figure 2A). Randomly distributed flowers were collected from the trees in May, immediately before anthesis. They were placed into petri dishes and stored at room temperature for about two hours. After that, the viability of the pollen was examined. The flowers from each tree were kept separate from the other flowers. In the phenologically mixed populations of flowers, care was taken to select recently open or opening flowers with petals recently separated, with the ovary visible and yellow, turgid and intact anthers. Flowers were removed from the same trees each year.

Pollen grains were extracted directly from dehiscent anthers using a sterile lancet and collected in a petri dish for each tree. The viability of the pollen grains was estimated by means of the FCR test procedure, one of the main methods currently available for evaluating pollen quality (Heslop-Harrison & Heslop-Harrison 1970). This method mainly tests the integrity of the plasmalemma of the vegetative cell. The fluorescein-diacetate penetrates the plasmalemma of living cells, where it is hydrolysed. The hydrolysis provokes a secondary fluorescence with green or brilliant greenish-yellow colouration under ultraviolet light. The intensity of the fluorescence is associated with both the activity of the esterase and the degree of integrity of the cell. The brilliant green fluorescence indicates full enzymatic activity and the intactness of the cell membrane (viable pollen grain; Figure 2B). Opacity is associated with low or null enzymatic activity (non-viable pollen grain). A Leica TCS-SL microscope with a 50 W mercury lamp at blue excitation (488 nm) was used for pollen observations. Between 150 and 200 pollen grains per slide were counted, regardless of their fluorescence. The grains that fluoresced bright green or at the wavelengths of 488 nm were also counted to determine the percentage of viable pollen. The test was repeated three times for each tree.

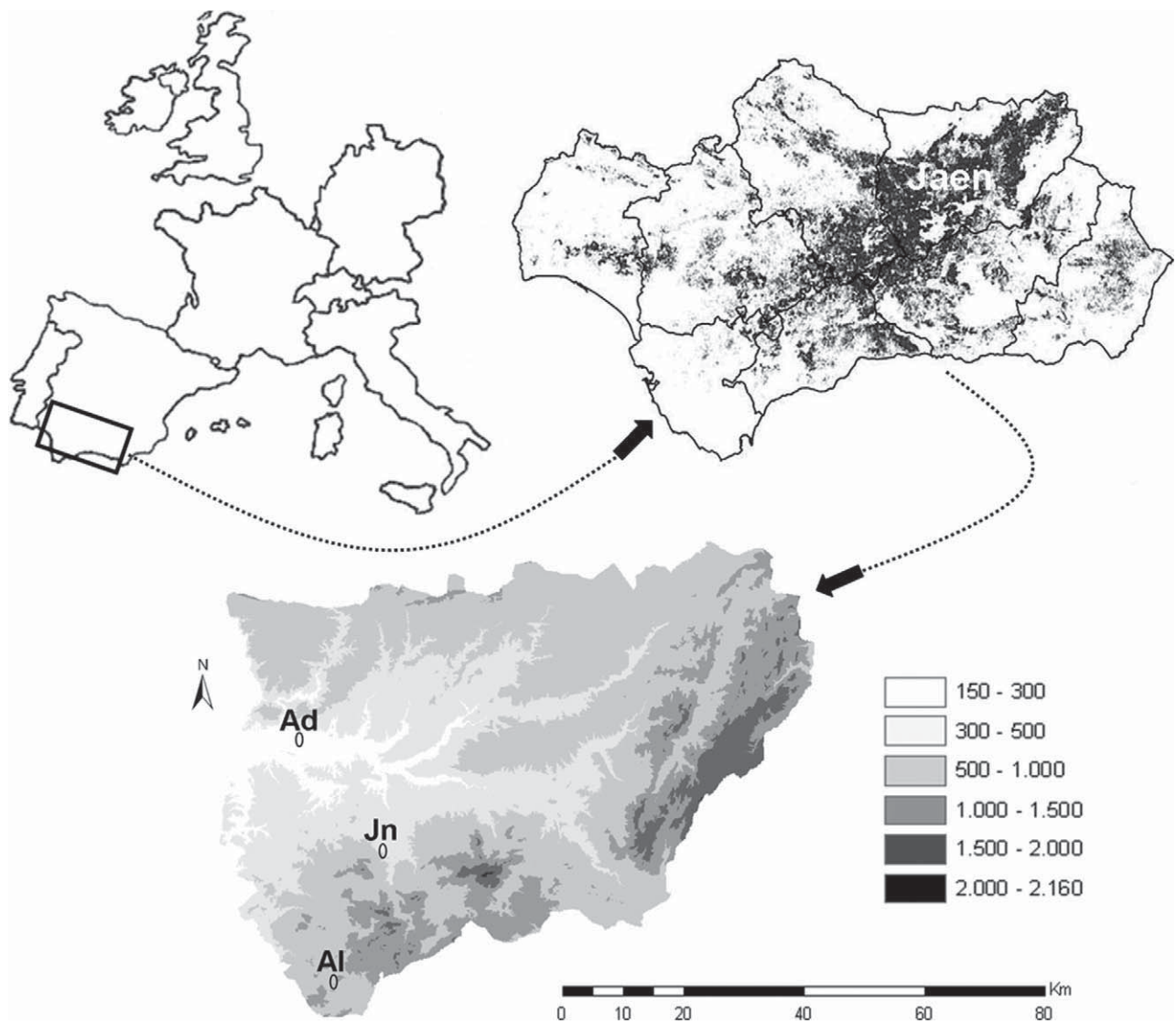


Figure 1. Location of the three study sites in the province of Jaen, Spain. Ad: Andujar; Jn: Jaen; Al: Alcala la Real. The numerical ranges refer to altitudinal ranges in metres.

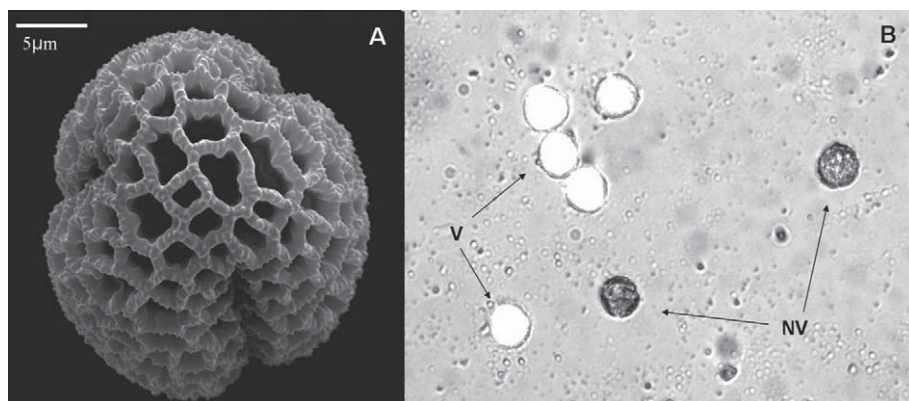


Figure 2. (A) *Olea europaea* cultivar 'Picual' pollen grain observed under electronic microscopy. (B) Viable (V) and non-viable (NV) pollen grains observed under fluorescence microscopy.

Viability values (i.e. percentage of pollen viable of all the pollen grains observed) were classified as high (> 50%), medium (25–50%) or low (< 25%) (Lousert and Brousse 1980).

2.3. Pollen longevity

Viability was also tested on the first day of anthesis (day 0) and 1, 3, 7, 10 and 15 days after anthesis to determine pollen longevity, i.e. the period of time during which pollen grains remain viable. The pollen grains were removed during this period from the flowers stored in the petri dishes, which were kept at 21–25°C. This temperature is close to the mean temperature during the olive pollen season in the study area (Aguilera & Ruiz Valenzuela 2009). Similarly, 150–200 pollen grains per slide were counted; the test was repeated three times for each tree per each day of evaluation.

2.4. Statistical analysis

Viability percentages were calculated by means of the following formula: Percentage of viable pollen = (number of pollen viable/total observed pollen grains) × 100.

Data on the differences between the study areas, collection years and pollen viability were examined using analysis of variance (factorial ANOVA). In this analysis, location and year were included to determine whether these factors and their interaction have a significant influence on the parameter being measured. A 95% confidence level was used.

3. Results

Percentage of viability was not significantly different between olive-growing areas ($F = 2.989$, $p = 0.055$).

However, it differed significantly among the three years ($F = 7.736$, $p = 0.000$). These differences were mainly due to the average percentage of viability recorded in 2007 for the trees of Jaen (64.86% $\pm$ 7.68%) (Table 1), a figure higher than the 51.69% ($\pm$ 19.14) found in pollen from trees in Andujar and the 49.98% ($\pm$ 11.27) in pollen of Alcala la Real (Figure 3). The percentages of viability in 2008 ranged from 41.35% ($\pm$ 16.16) to 48.06% ($\pm$ 13.47). Similar average values were obtained in 2009, with percentages ranging from 40.81% ($\pm$ 7.71) to 50.36% ($\pm$ 14.94).

During the overall study period, the average percentage of viability recorded in the pollen grains from trees in Andujar was 44.62% ($\pm$ 6.13). The highest values were found in trees from Jaen and Alcala la Real, with average viability percentages of 51.99% ($\pm$ 11.72) and 49.47% ($\pm$ 1.23), respectively. Interestingly, in two of the three years (2008 and 2009) the trees from Alcala la Real contained the most viable pollen, while the trees from Andujar contained the less viable pollen.

The average percentage of viability was between 44% and 52% on day 0, at the time of anthesis (Figure 4). The highest average percentages of viable pollen were observed on day 1, with percentages ranging from 47% to 57%. The percentages of viability decreased significantly from day 1 to day 3 after anthesis ($F = 49.303$, $p = 0.002$). Percentages of viable pollen ranged from 25% to 30%. After day 3, percentages dropped slowly until day 7 after anthesis, with average percentages of viability ranging from 21% to 22%. After day 7, viability percentages dropped from 21% to below 10% on day 15 after anthesis.

4. Discussion

Although the study sites were distributed on an altitudinal gradient and there were consequently different microclimatic conditions, the percentages

Table 1. Percentage of *Olea europaea* cultivar ‘Picual’ pollen viability for each tree, year and study area. The average percentages of pollen viability were statistically analysed by Factorial ANOVA. Different letters indicate statistically different values ($p < 0.05$).

Study area	2007 average	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5
Andujar	51.69 $\pm$ 19.14 ^a	73.69	29.52	49.55	68.41	37.30
Jaen	64.86 $\pm$ 7.680 ^b	65.98	52.13	65.88	67.35	72.97
Alcala la Real	49.98 $\pm$ 11.27 ^a	51.41	36.83	41.58	65.54	54.52
Study area	2008 average	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5
Andujar	41.35 $\pm$ 16.16 ^a	43.75	22.96	59.29	54.12	26.64
Jaen	41.91 $\pm$ 12.38 ^a	32.74	49.64	53.52	24.93	48.74
Alcala la Real	48.06 $\pm$ 13.47 ^a	61.26	33.21	46.00	62.56	37.26
Study area	2009 average	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5
Andujar	40.81 $\pm$ 7.710 ^a	52.65	33.03	42.67	40.69	35.00
Jaen	49.21 $\pm$ 14.91 ^a	62.27	58.53	52.73	24.33	48.20
Alcala la Real	50.36 $\pm$ 14.94 ^a	66.26	35.21	37.26	65.56	47.50

^{a,b}Indicate statistical differences in the average percentages of pollen viability comparing years and study areas.

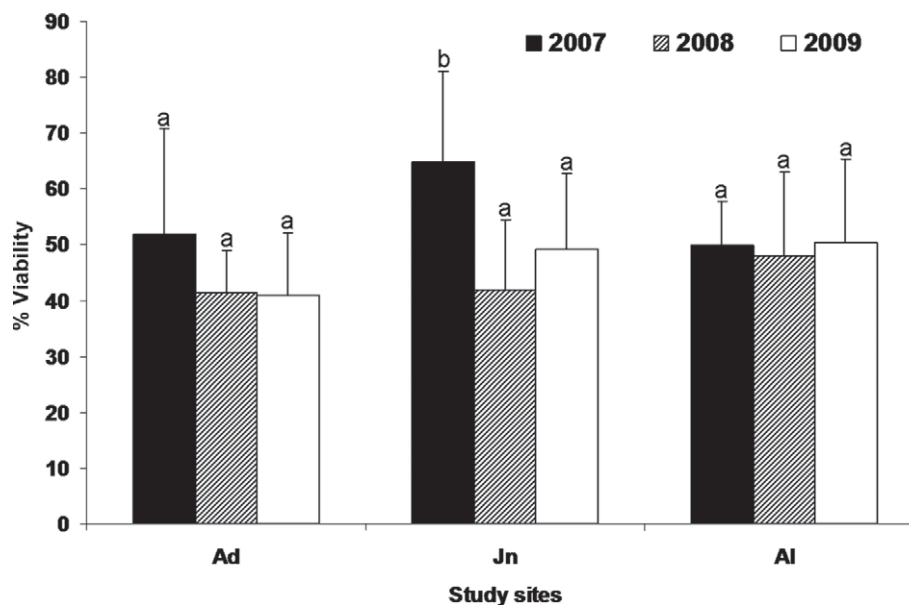


Figure 3. Viability percentages for each year and study area. Ad: Andujar; Jn: Jaen; Al: Alcala la Real. Different letters indicate statistically different values ($p < 0.05$).

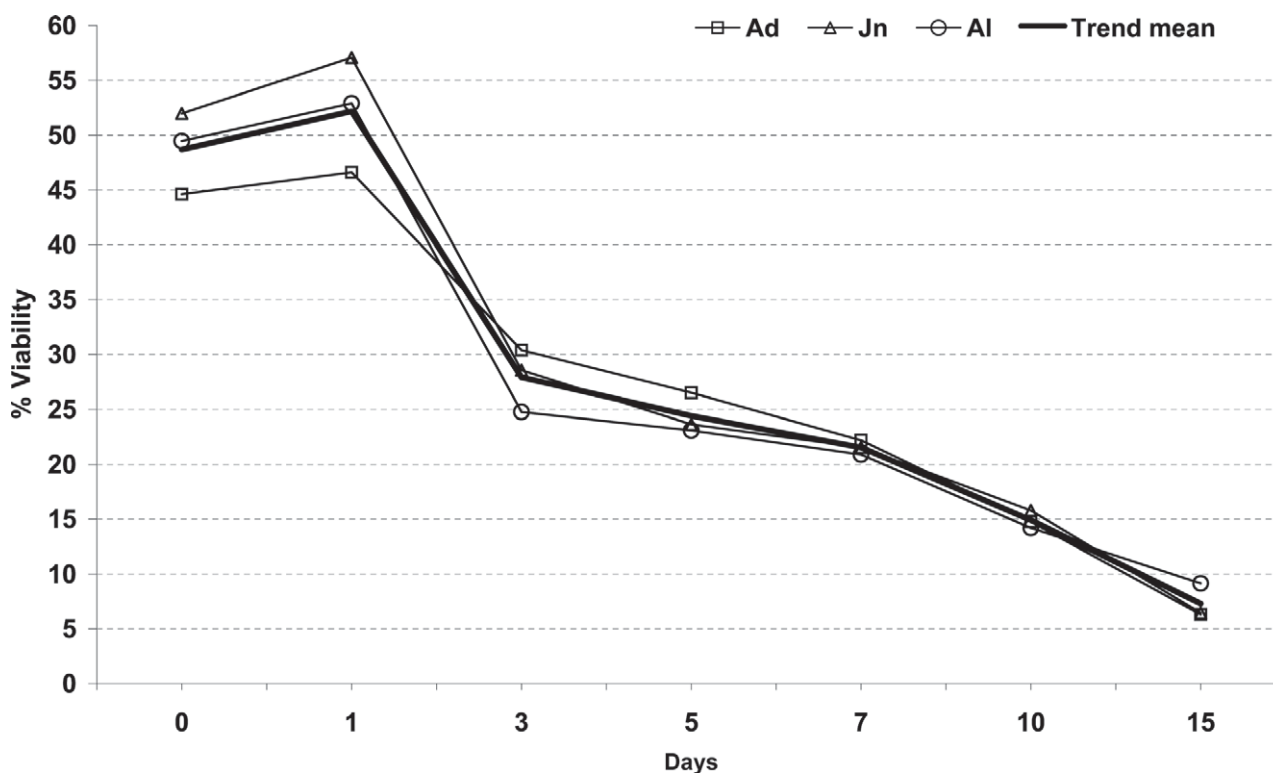


Figure 4. Average time trends (in days) in the viability of *Olea europaea* cultivar 'Picual' pollen grains.

of viability obtained in this research did not show any geographical variability. In general, the percentage of viability did not differ throughout the years. However, the olive groves situated at higher altitudes with lower temperatures and higher rainfall rates showed the highest average percentages of viable

pollen. This could be interpreted as a reproductive strategy to ensure fertilisation, since a greater percentage of pollen viability could compensate the shorter flowering period of the olive groves situated at higher altitudes (Aguilera & Ruiz Valenzuela 2009).

With an average value of 49%, the viability of the pollen in the cultivar 'Picual' could be classified as medium-range. Our data differ from those of Rovira & Tous (2005), who recorded only 35.4% of viability by means of the FCR test. This fact could be due to different sucrose availability in the media, the phenological state of the flowers at the moment of collection, the state of the anthers or even storage conditions.

The pollen grains of the cultivar 'Picual' show the highest viability 24 hours after anther dehiscence, most likely within a few hours after pollination. This phenomenon is related to the success of fertilisation which depends not only on pollen production, but also on pollen viability and stigma receptivity. The pollen grains should have the maximum potential when they land on the stigma, because both the stigma and the ovular receptivity last only one or two days (Rapoport 2008; Serrano et al. 2008).

Viability declined rapidly with time. The longevity of olive pollen viability was about seven days. However, the first three days after anthesis were certainly the most important for fertilisation because this is the time when the pollen grains were viable in medium-high percentages. The rapid decrease in pollen viability could be related to the loss of permeability to water of the intine during storage, with the subsequent reduction of pollen hydration (Ferri et al. 2008). A rapid decline in pollen viability may considerably reduce effective fertilisation and could negatively influence olive fruit production. For this reason, and from an agronomic point of view, it is necessary to determine both the olive pollen viability and longevity to improve fruit yields, considered in future studies and by olive growers.

5. Conclusions

The results of this study provide essential information on the reproductive biology of the olive cultivar 'Picual'. The pollen viability of this cultivar, assessed by the enzymatic activity and the integrity of the plasmalemma, could be classified as medium-range although it declines rapidly with time. The initial three days after anthesis are the most important for the fertilisation of the ovaries. These results could be used in breeding programmes or studies on the fertilisation process.

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Author biographies

Fátima Aguilera is Dra. in Biología for the University of Jaén, Spain. Her research is based on different aspects of reproductive development in the olive (*Olea europaea* L.),

a native Mediterranean species. She has published papers in numerous publications, both national and international, concerning olive pollen emission and dispersion in the southern Iberian Peninsula, which show important altitudinal fluctuations conditioned by both the location of the olive grove and for the microclimatic conditions associated with this factor. Furthermore, her investigation on phenological development is showing interesting results, such as their work on the study of pollen production and pollen viability. Dra. Aguilera are currently working at the University of Perugia, Italy, with a post-doctoral grant from Fundación Ramón Areces (Madrid, Spain). She is analysing phenological data to identify how olive crops have adapted to their local climate and how they respond to climatic change.

Luis Ruiz Valenzuela is Dr. in Biología for the University of Granada, Spain. He is currently working as Professor in the Department of Animal Biology, Plant Biology and Ecology of the University of Jaén. His research is based on allergenic aspects because olive pollen is the main cause of pollinosis in the Mediterranean area, provoking seasonal allergic rhinitis and bronchial asthma among the population.

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