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Effective pollination period for 'Manzanillo' and 'Picual' olive trees

By J. CUEVAS[1]*, V. PINILLOS[1] and V. S. POLITO[2]
[1]Departamento de Producción Vegetal, Universidad de Almería, La Cañada de San Urbano, s/n 04120 Almería, Spain
[2]Department of Plant Sciences, 1 Shields Ave., University of California Davis, 95616 Davis, CA, USA
(e-mail: jcuevas@ual.es) *(Accepted 16 January 2009)*

SUMMARY
The effective pollination periods (EPP) for 'Manzanillo' and 'Picual' olive trees have been determined in two consecutive years in irrigated orchards in California and Spain. The duration of the EPP was variable between year and cultivar. Fruit set declined gradually in response to sequential cross-pollination. Consistent differences with respect to the maximum levels of initial fruit set occurred between 4 – 6 d after anthesis (DAA) in 'Manzanillo', and between 8 – 14 DAA in 'Picual'. Fertilisation was first observed on day-2 after cross-pollination in both cultivars. Ovule longevity, determined by fluorescence microscopy, appeared to last > 14 d in both cultivars. The stigmas remained receptive for > 8 d. Estimates of the duration of the EPP, based on analyses of its components, were longer than those indicated by the declines observed in fruit set, suggesting that other factors such as the suitability of the style to support pollen tube growth may limit the duration of the EPP. These results suggest that self-incompatibility may be a more important factor than a short EPP in limiting fruit set in 'Picual', as well as in 'Manzanillo' olive trees.

'Manzanillo' is the main table olive cultivar grown in California, although, in some locations, its productivity has declined significantly. Lack of an effective polliniser may explain such losses. Self-incompatibility in olive has remained controversial, although most olive orchards in Spain and California do not include pollinisers. However, there is growing recognition of the beneficial effects of cross-pollination (Cuevas *et al*., 2001; Lavee and Datt, 1978; Moutier, 2002; Sibett *et al*., 1992). 'Picual' is the main olive oil cultivar grown in Spain, where it is mainly planted in monocultivar orchards. Under these conditions, no apparent problems in productivity have been attributed to poor pollination.

A short life-span of flowers may also diminish fruit set. Frequent fruit set failure in apple led Williams (1965) to develop the concept of the effective pollination period (EPP). EPP is defined as the period during which pollination can produce fruit set. EPP incorporates stigma receptivity, pollen-tube growth, and ovule longevity, and was proposed to be the result of ovule longevity minus the time required for pollen-tube growth to reach the egg apparatus, as long as stigma receptivity is non-limiting. The duration of the EPP can be estimated by fruit set in response to sequential pollination and by direct analyses of the components of the EPP (i.e., ovule longevity, fertilisation time and stigma receptivity). In some olive cultivars, the EPP has been found to vary between 4 – 8 d (Arzani and Javady, 2002; Bini, 1984; Villemur *et al*., 1984), suggesting that the duration of the EPP may limit fruit set. EPP has not been measured in 'Manzanillo' or 'Picual' olives, and its importance for losses in productivity has not been explored.

*Author for correspondence.

MATERIALS AND METHODS

Plant material

The experiments were conducted on 'Manzanillo' olive trees growing at Wolfskill orchard, University of California, Davis, CA, USA and on 'Picual' olive trees in two different orchards in Southern Spain ("Venta del Llano" Experimental Station, Jaén and "Venta los Yesos", Almería). All trees were regularly managed following standard commercial practices. In each experimental year, four trees were selected for their uniform size and high level of flowering. The experimental design was a randomised complete block in which each tree acted as a block and replication.

Measurements

The duration of the EPP was inferred by fruit set resulting from sequential pollination and by analyses of the components of the EPP. Initial fruit set for each date was calculated as the percentage of pollinated flowers that became fruits 21 d after pollination. Approx. 100 hermaphrodite flowers were used for each pollination date. Consistent flower age was assured by removing all open flowers 1 d before anthesis of the experimental flowers, and by eliminating closed flowers the next day. Unwanted cross-pollination before the scheduled date was prevented by bagging. 'Manzanillo' flowers were hand-pollinated with 'Sevillano' pollen at anthesis, and 1, 2, 3, 4, 6, 8, or 11 d after anthesis (DAA). 'Picual' flowers were pollinated with 'Hojiblanca' pollen at anthesis, and 2, 4, 6, 8, 10, 12, or 14 DAA. Cross-compatibility between cultivars had previously been confirmed (Cuevas *et al*., 2001). 'Sevillano' and 'Hojiblanca' pollen viability had been assessed previously by fluorochromatic reaction (Heslop-Harrison and Heslop-Harrison, 1970). Flower emasculation was not performed. Instead, 100 hermaphrodite flowers per tree remained bagged to

determine the level of fruit set in self-pollinated controls. Fruit set was determined 21 DAA, before fruitlet abscission due to competition for nutrients could mask the results.

The components of the EPP (i.e., stigma receptivity, fertilisation date, and ovule longevity) were determined using fluorescence microscopy. To determine stigma receptivity, 20 flowers (i.e., five on each tree) per sampling date were emasculated at the white balloon stage and covered with tissue-paper bags to prevent pollination. The day after emasculation was considered the date of anthesis. Flowers were then hand-pollinated on various dates after anthesis, and collected 1 d later. Stigma receptivity was expressed as the percentage of flowers that supported pollen adhesion. Ovule viability was determined using the onset of aniline blue fluorescence as an indicator of ovule senescence (Cuevas *et al*., 1994) on different samples of 20 virgin flowers (i.e., five on each tree) per sampling date. The percentage of flowers with at least one of its four ovules remaining viable (i.e., non-fluorescent) was then calculated at different times after anthesis. The duration of ovule longevity was the last day on which at least 50% of flowers contained viable ovules. Fertilisation date was calculated using another sample of 20 flowers per sampling date that were cross-pollinated at anthesis and collected on various dates after pollination. In this case, the flowers were not emasculated. The climatic conditions during all experiments were recorded from nearby weather stations. The temperatures are shown in Table I.

TABLE I
Maximum, minimum, and average temperatures during flowering in each experimental year

		Temperature (ºC)		
Cultivar	Year	Maximum	Minimum	Average
'Manzanillo'	1994	30.8	11.2	20.7
	1995	28.7	11.1	18.6
'Picual'	2000	28.9	13.0	20.3
	2001	21.6	8.8	15.5

Data analysis

Fruit set data were compared by analyses of variance, and the separation of means was achieved by Tukey's tests using SAS software (SAS Institute Inc., Cary, NC, USA). Percentages were previously transformed to arcsine square root.

RESULTS

Fruit set

Both olive cultivars ('Manzanillo' and 'Picual') behaved as being strongly self-incompatible. The mean level of fruit set in bagged self-pollinated flowers was approx. 6% and 2% in 'Manzanillo' and 'Picual', respectively (Figure 1; Figure 2). These values were six-

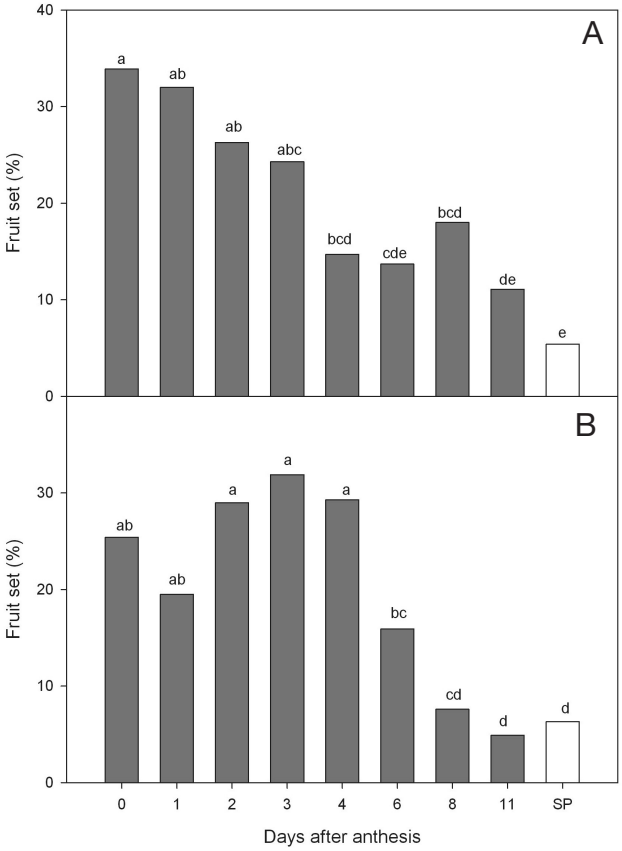

FIG. 1
Initial fruit set percentages from pollinations on increasing days after anthesis (DAA) in 'Manzanillo' trees in 1994 (Panel A) and in 1995 (Panel B). SP; percentage of fruit from flowers in bagged shoots. Data were subjected to analysis of variance. Percentages were previously transformed to arcsine square root. Mean separations by Tukey's test at $P < 0.05$.

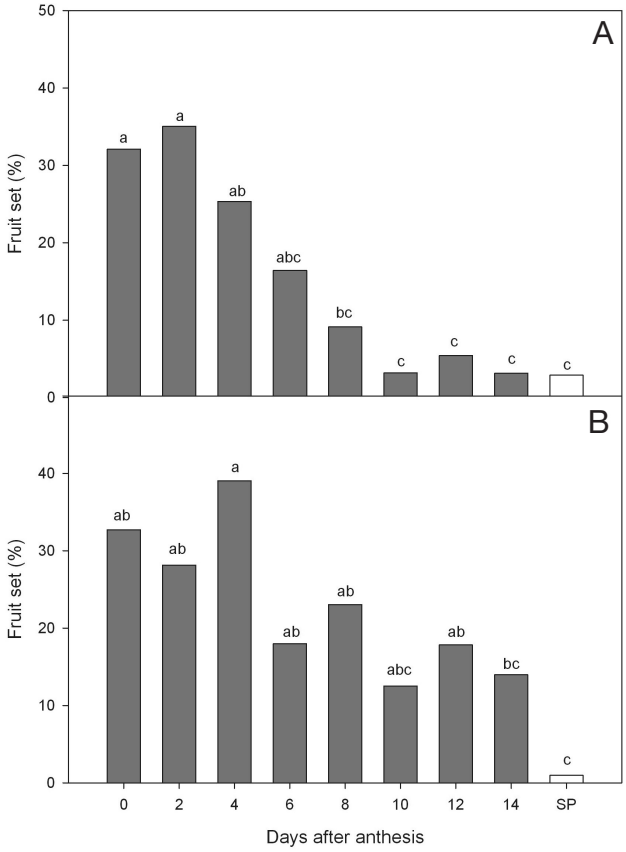

FIG. 2
Initial fruit set percentages from pollinations on increasing days after anthesis (DAA) in 'Picual' trees in 2000 (Panel A) and in 2001 (Panel B). SP; percentage of fruit from flowers in bagged shoots. Data were subjected to analysis of variance. Percentages were previously transformed to arcsine square root. Mean separations by Tukey's test at $P < 0.05$.

TABLE II

Percentages of flowers with receptive stigmas (SR) and fertilised ovules (F), and percentages of fertile flowers with at least one viable ovule (OL a) and of viable ovules (OL b) on increasing dates after anthesis (DAA) in 'Manzanillo' olive trees in 1994 and 1995

Days after anthesis	1994				1995			
			OL				OL	
	SR	F	a	b	SR	F	a	b
0	60		100	88	87		95	82
1	79	6			71	5		
2	55	26			81	10		
3	90	21			89	50	89	87
4	67	47			68	45		
5							90	85
6	65	35	95	88	50	40		
7								
8	80	55	94	81	47		47	25
10							85	75
11		55	94	81		47		
12							88	79
14			89	76			53	27
16			66	40				
18			25	18			47	34

and 20-times lower than the maximum level of fruit set achieved using cross-pollination.

In 1994, the maximum fruit set (34%) in 'Manzanillo' was achieved following pollination at anthesis (Figure 1). This level decreased gradually, becoming significantly lower (22%) with pollination at 4 DAA. In 1995, the maximum fruit set (32%) was attained from pollinations carried out ≤ 4 DAA. Fruit set declined abruptly with pollination at 6 DAA and, 2 d later, equalled the levels achieved by self-pollination (6%).

In 2000, the maximum fruit set (33%) in 'Picual' was achieved from pollinations carried out within 2 DAA, and this percentage decreased with later pollinations, becoming significantly different 8 DAA (Figure 2). In 2001, the maximum fruit set (40%) was obtained from pollination at 4 DAA, and decreased gradually, although it did not become significantly lower until pollinations at 14 DAA.

Estimates of EPP using sequential pollination were therefore 3 d and 4 d for 'Manzanillo', and 6 d and 12 d for 'Picual', in the first and second years, respectively.

Ovule longevity

Ovule longevity was prolonged in emasculated flowers of both cultivars, in both experimental years (Table II; Table III). Olive flowers have four ovules. Typically, only one of the four ovules is fertilised and develops into a seed. In 1994, more than 50% of 'Manzanillo' flowers had at least one viable ovule at 16 DAA. On this date, only 40% of the total ovules were viable. Two days later, the percentage of flowers with at least one viable ovule decreased to 25%, and only 18% of all ovules were still viable. In 1995, we considered ovule longevity to be 14 d since, at this time, 50% of flowers still contained viable ovules. In the case of 'Picual', ovule longevity extended beyond the experimental end date (16 d and 20 d) in 2000 and 2001. However, the percentage of flowers with some senescent ovules increased after 6 DAA. In 2000, at 10 DAA, 61% of flowers had senescent ovules (Table III). In 2001, the percentage of flowers with senescent ovules did not exceed 50% until 16 DAA.

Stigma receptivity

Stigma receptivity was also prolonged in 'Picual' trees. In this cultivar, 67% and 100% of stigmas were still receptive at 16 and 20 DAA in 2000 and 2001, respectively (Table III). In 'Manzanillo', stigma receptivity lasted for ≥ 8 d, the last time stigma receptivity was checked. At this time, 80% and approx. 50% of stigmas remained receptive in 1994 and 1995, respectively (Table II).

Fertilisation time

In both cultivars, fertilisation was first observed 1–2 d after cross-pollination. In 'Manzanillo', approx. 50% of flowers were fertilised after 3 – 4 d, depending on the year (Table II). In the case of 'Picual', the majority of flowers were fertilised 8 d after pollination (Table III).

TABLE III

Percentages of flowers with receptive stigmas (SR) and fertilised ovules (F), and percentages of fertile flowers with at least one viable ovule (OL a) and of viable ovules (OL b) on increasing dates after anthesis (DAA) in 'Picual' olive trees in 2000 and 2001

Days after anthesis	2000				2001			
			OL				OL	
	SR	F	a	b	SR	F	a	b
0	100	0	100 (0)*	100	100	0	100 (0)*	100
2		25				20		
4		32				35		
6	100	29	100 (39)	87	100	45	100 (10)	98
8		61	96 (44)	80		67	100 (15)	95
10		67	100 (61)	72		85	100 (20)	94
12	100	71	96 (65)	76	90	90	100 (50)	81
16	67		100 (87)	68			95 (70)	68
20					100		95 (65)	68

*Values in parentheses are the percentages of flowers with senescent ovules.

DISCUSSION

In the present study, we have determined the EPP, and its components, in 'Manzanillo' and 'Picual', the most common olive cultivars grown in California and Spain, respectively. The duration of the EPP varied between cultivar and year. In 'Manzanillo', under Californian conditions, the EPP lasted for 3 – 4 d, depending on the season; whereas, in 'Picual', under Spanish conditions, the EPP was longer and more variable, lasting between 6 – 12 d. The duration of the EPP in fruit trees is highly variable, depending on species, cultivar, and environmental conditions, and ranges from 2 d to more than 1 week (Sanzol and Herrero, 2001). Our results agree with those in other reports on the EPP in olive, that ranged from 4 – 8 d (Arzani and Javady, 2002; Bini, 1984; Villemur *et al*., 1984).

Variations in the EPP between and within cultivars have been observed in many species (Brevis *et al*., 2006; Burgos *et al*., 1991; Guerrero-Prieto *et al*., 1985; Keulemans and van Laer, 1989; Williams, 1965). In olive, Villemur *et al*. (1984) reported a longer EPP in 'Picholine' than in 'Luques' under the same conditions in two different years. They attributed the difference to shorter ovule longevity in 'Luques'.

Environmental conditions, especially temperature, and internal flower factors can influence the EPP greatly (Sanzol and Herrero, 2001). The high variation in the duration of the EPP found in 'Picual' seems to be related to the different temperatures during bloom. In 2001, with a longer EPP, the average temperature in the orchard during the bloom period was nearly 5°C lower than in 2000. Temperature has a clear effect on ovule longevity and pollen tube growth, as well as on stigma receptivity. High temperatures caused a reduction in ovule longevity in pear and apple (Tromp and Borsboom, 1994; Vasilakakis and Porlingis, 1985), and in stigma receptivity in apricot and kiwi (Egea and Burgos, 1992; Gonzalez *et al*., 1995). Cuevas (1992) reported longer ovule longevity in 'Arbequina' olives at 20°C than at 25°C. In 'Picual', no differences were found between years for estimates of ovule longevity, based on the percentage of flowers with at least one viable ovule. However, there was a more rapid increase in the percentage of flowers with senescent ovules in 2000 than in 2001 (Table III). Other factors, especially flower quality, may be involved in the different EPP values found between years.

In many species, good correlations have been found between the percentage of fruit set following delayed pollination, and microscopic analyses of ovule development, pollen tube growth, and stigma receptivity. This made it possible to determine the limiting factor for the EPP (Sanzol and Herrero, 2001). However, the duration of the EPP estimated here from delayed pollination experiments was shorter, in all cases, than estimates obtained from the differences between ovule longevity and the time-lag between pollination and fertilisation, or the period of stigma receptivity. In both 'Manzanillo' and 'Picual', cross-pollen tube growth was rapid, and the first fertilised flowers were observed 1 – 2 d after pollination. Relatively rapid pollen tube growth (3 – 4 d) has also been reported in other olive cultivars under cross-pollination (Arzani and Javady, 2002; Villemur *et al*., 1984). Similarly, stigma receptivity was found by Villemur *et al*. (1984) to be prolonged; thus, neither variable seems to be the limiting factor for the EPP. Ovule longevity, however, has not been determined in any previous studies on EPP in olive. Villemur *et al*. (1984) inferred ovule longevity from fruit set data following delayed pollinations. They assumed that the reduction in fruit set was due to ovule senescence, and estimated that ovule longevity lasted 8 d and 12 d in 'Lucques' and 'Picholine' olives, respectively. Our results, based on the onset of aniline blue fluorescence in ovules, show extended periods of ovule longevity. In 'Picual', this extended beyond the duration of the experiment (16 – 20 d). Fernández-Escobar *et al*. (2008) used the aniline blue technique to estimate ovule longevity in 'Picual' over 2 years, following different nitrogen fertilisation treatments. They reported at least 50% of ovaries with viable ovules 10 DAA in the first year, and 20 DAA in the second year. Using the same procedure, Cuevas (1992) found more than 50% of flowers with viable ovules 22 DAA in 'Arbequina' olive. This author considered that this result could be an overestimation of ovule longevity, due in part to the difficulty of distinguishing the beginning of senescence.

Another possibility is that the style receptivity period, or style suitability period, as defined by Egea and Burgos (1992), limits the EPP. The decrease in fruit set following delayed pollination could be due to a senescent style that prevents the pollen tube from reaching and fertilising the ovule, even though viable ovules are still present. This may explain the discrepancy between estimations of the duration of the EPP observed here. In durian, a significant correlation was found between the inhibition of pollen tube growth in the style and the reduction in fruit set (Honsho *et al*., 2007).

Self-incompatibility in 'Picual' and in 'Manzanillo' olive was previously reported (Cuevas and Polito, 1997; Cuevas *et al*., 2001), and is confirmed here as both cultivars had limited fruit set under self-pollination. Therefore, in 'Picual' and 'Manzanillo' orchards, self-incompatibility rather than a short EPP seems to be the main constraint for fruit set. However, a relatively brief EPP, as found in 'Manzanillo', emphasises the importance of selecting polliniser cultivars with bloom periods that consistently overlap that of the main cultivar.

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