Article

Profitability of Artificial Pollination in ‘Manzanillo’ Olive Orchards

Alberto Sánchez-Estrada 1,2 and Julián Cuevas 2,*

1 Centro de Investigación en Alimentación y Desarrollo, Coordinación de Alimentos de Origen Vegetal A.C.
Km 0.6 Carr. a La Victoria, Hermosillo C.P. 83304, Sonora, Mexico; aestrada@ciad.mx
2 Department of Agronomy, Campus de Excelencia Internacional Agroalimentaria (ceiA3), University of Almería, La Cañada de San Urbano, s/n 04120 Almería, Spain
* Correspondence: jcuevas@ual.es; Tel.: +34-50-215599

Received: 2 April 2020; Accepted: 30 April 2020; Published: 4 May 2020

Abstract: The fruit set in monovarietal ‘Manzanillo’ olive orchards is significantly increased under cross-pollination. This response lead to pollination designs including pollinizer selection, the number of pollinizer trees per hectare and their distribution in the orchard. However, the assignment of a substantial area to pollinizers of lesser commercial value might decrease profits. The strong influence of variable climates on the overlap of the blooming phenology of ‘Manzanillo’ and its pollinizer, and on pollen production and dispersal, are also notable risks. Artificial pollination is a feasible alternative to pollination designs, especially for wind-pollination crops such as olives. Here, we present the effects of treatments with different number (zero, one, two or four) of mechanical applications of ‘Barouni’ pollen on fruit set, size, yield, and cost–benefit ratios in heavy- and light-flowering trees of ‘Manzanillo’ trees situated in monovarietal orchards in Sonora, Mexico. Our results showed that, in “on” years (seasons where most trees display abundant flowering), a larger number of cross-pollen artificial applications increased more the final fruit set, yield and, hence, the profits. Fruit size was scarcely affected by the number of applications, although treatments with lower fruit sets had a higher proportion of large-sized fruit and less fruit of petite size. Despite its higher costs, the higher increase in yield made it more profitable to apply cross-pollination four times throughout the blooming period. On the other hand, no significant differences were observed among treatments, regardless of the number of pollinations, in the “off” season (the season in which most trees had a light flowering level).

Keywords: Olea europaea; monovarietal orchards; fruit set; fruit size; yield; cost–benefit ratio

1. Introduction

Olives (Olea europaea L.) strongly depend on cross-pollination to obtain high fruit set levels [1–3]. This dependence is clear for ‘Manzanillo’ [4], the most extensively grown and esteemed table olive cultivar in the world [5]. Monovarietal orchards of ‘Manzanillo’ in isolated locations, without the benefits of free cross-pollination, suffer from severe pollen deficits [6–9], significantly reducing their flower fertilization and fruit set [4,10]. The usual measure to overcome pollen deficits in tree crops is to design plantations with the right number of pollinizers (pollen donor trees), with the right distance from the main variety, and in the right patterns. The chosen pollinizer has to be compatible with the main variety and accomplish requisites such as presenting a large overlap in the phenological stage of blooming, being a regular bearer (not alternating much), and ideally having the same purpose (table or oil) [11,12]. Pollination designs are not exempt from failures. Low flowering levels and poor pollen viability in the pollinizer and a lack of flowering overlap between the main variety and the pollinizers are risks that are always present [13]. Assigning a large proportion of the orchard...
area (from 10% to 50%) and inputs to a less valuable cultivar acting as a pollen donor can reduce profitability too [14].

Artificial pollination (AP) is defined as the biological or mechanical application of compatible pollen previously harvested, stored and evaluated [15]. AP can be an alternative to pollination designs, since it reduces the uncertainty derived from natural pollination, avoiding, in some cases, the need to plant pollinizers [13,14,16,17]. There is evidence of the use of AP dating as far back as the Assyrian Empire and Ancient Egypt (1700-800 BCE). Other ancient cultures also used this method [15,18]. The aim of AP is to solve pollination problems that arise for natural reasons, whether climatic, physiological, morphological and/or due to the lack of pollinizers [19–22]. Commercial reasons such as improving fruit set, correcting planting design mistakes and enhancing product quality (mainly fruit size) are also important motives. AP has been successfully tested in various entomophilous species such as vanilla [20], apple [23], kiwi [13] and custard apple [24], but it is often more effective in anemophilous crops such as date palm [25], pistachio [26], walnut [27], hazelnut [28] and olive [14,15].

AP includes several important steps to achieve commercial success. An economical pollen harvest that involves the optimal moment of pollen collection and easy handling [29], long term pollen storage [30,31], reliable pollen viability measurement [32,33] and inexpensive mechanical application. For this last step, the best date, number of applications and pollen dose must be determined and its cost assessed [13–15,34]. Repeated applications increase the cost and can make AP prohibitive. Cost–benefit ratios must be calculated for different scenarios.

In this report, we compare the profitability of treatments differing in the frequency of pollen applications in terms of fruit set, fruit size (value) and yield. We also estimated the cost–benefit ratio in order to determine the treatment that is financially optimal in “on” and “off” ‘Manzanillo’ trees.

2. Materials and Methods

2.1. Plant Material and Orchard Location

Artificial pollination experiments were carried out in 2017 and 2018 in commercial ‘Manzanillo’ monovarietal orchards located in Caborca municipality, Sonora, Mexico (latitude 30°49′54.8″ N, longitude 112°54′039.9″ W) at 200 m.a.s.l. According to Ruiz-Corral et al. [35], the climate of the area is of the Sonoran Desert type. The precipitation is low, with an annual mean rainfall of 103.8 mm, mostly during summer and winter. Total rainfall in 2017 was 127 mm, while it was 164 mm in 2018. The seasonal distribution of rainfall was 23 mm for winter (Jan–March), just 2 mm in spring (Apr.–June), 68 mm in summer (July–Sept.) and 34 mm in autumn (Oct.–Dec) during 2017. In 2018, the seasonal rainfall distribution was 32 mm, 1 mm, 92 mm and 39 mm, for the Jan-March, April-June, July-September and October-December, three-month periods, respectively. The average annual air temperature in the area is 22.7 °C, with January being the coldest month (mean 13.4 °C) and July the hottest (mean 33.2 °C).

In the “on” year (2017), the season in which most trees had abundant flowering, the trial was carried out in a 15-year-old ‘Manzanillo’ orchard. Trees were vase-trained and spaced at 8 × 10 m in an isosceles triangular layout design. In the “off” year (2018) season in which most trees had a light flowering level, the trial was performed on 20-year-old ‘Manzanillo’ vase-trained trees planted at a distance of 10 × 10 m. The soil was, in both orchards, manually fertilized by applying 1 kg of N per tree in the form of urea split in two applications carried out in February and June, and 300–400 g tree⁻¹ of K, applied as potassium sulfate, in February, before irrigation. Orchard management included monthly downstream irrigation. Weeds, mostly grass and goosefoot, were controlled by tillage. Olive fly, the main pest in the experimental site, was controlled by using baited traps with attractants (1 tramp 20 ha⁻¹); copper solutions and Bordeaux mix were applied to control airborne fungal diseases.
2.2. Experimental Design and Treatments

Four homogenous trees per treatment were selected for the experiments, with high and low flowering levels, given the strong alternate bearing habit of this crop. In a qualitative ranked scale between zero (no flowering) and 10 (extremely high level of flowering), heavy flowering trees averaged a level of nine (year 2017), while the flowering level was four in the “off” season (year 2018). Olive blooms in panicles arising from lateral buds in 1-year-old shoots. We selected eight 1-year-old shoots per tree, functioning as subsamples within each replicate (tree). These shoots were located at the observer’s height around the periphery of the canopy. The number of panicles of each shoot was adjusted by hand before bloom to 16 and to 10 per shoot in “on” and “off”, respectively, to avoid the effects of different flowering loads on fruit set and fruit size. Olive is an andromonoecious species—that is, it forms male and hermaphrodite flowers, usually within the same panicle. Flowers in the experiment were kept under open pollination conditions, but in an isolated monovarietal orchard, where we previously had demonstrated a strong dependence on cross-pollination to obtain a sizable yield [9].

The experiment followed a completely randomized design. Three AP treatments were compared, differing in the number and timing of cross-pollen applications. AP treatments were one pollination at full flowering, two pollinations, first at the beginning of flowering and then at full flowering, and four pollinations, one at the beginning of flowering and then every 4 days. The beginning of flowering and full flowering dates were considered when 10% and 50% of open flowers were, respectively, observed [36]. Trees exposed to open pollination, but not receiving applications of ‘Barouni’ pollen, served as controls. In 2017, control trees were located 200 m from the point of AP applications. In 2018, we used, as controls, trees that were in a different plot 5 km distant from AP trees and 80 m away from trees of other varieties.

Pollen previously collected from ‘Barouni’ trees was applied following the recommendations of Cuevas and Pinillos [37]. The viability of this pollen was ascertained before use by the fluorochromatic staining test proposed by Heslop-Harrison and Heslop-Harrison [32] and slightly modified by Pinillos and Cuevas [33]. The percentage of fluorescent (potentially viable) pollen was 85.1% and 79.6% in 2017 and 2018, respectively. The pollen applied in 2018 was that collected in 2017 and stored at $-20^\circ\text{C}$ for one more year. The pollen, undiluted, was applied at a dose of 80 g ha$^{-1}$ for each application with a powder duster (Dustin mizer model 1212, Sioux Falls, SD, USA), directly to the tree canopy at 1–1.5 m distance.

2.3. Initial and Final Fruit Set and Size

Initial fruit set (IFS) 15 days after full bloom (dab) and final fruit set (FFS) 45 dab, as the number of fruits per panicle, were determined on tagged shoots. Seedless small shotberries were not counted. Near fruit maturity, at mid-August, all fruits present in tagged shoots were harvested and individually weighted in the lab. IFS and FFS and fruit weight at harvest were compared by an analysis of variance. The post-hoc Tukey test was used for the separation of means at a probability level of $p \leq 0.05$. Infostat software 2017e version (University of Cordoba, Cordoba, Argentina) was used for statistical analyses.

Yield (kg tree$^{-1}$) was estimated by taking into account the flowering intensity of each tree, the average flower number per panicle in ‘Manzanillo’ trees (9.74), recorded in a parallel experiment [9], the percentage of fertile panicles, i.e., possessing at least one hermaphroditic flower (88.7%), and final fruit set and mass (g) at harvest. Finally, the incomes derived from the different pollination treatments were compared based on yield and market price for each fruit size category [38]. The cost of purchased pollen, but not the labor costs of the applications, was taken into account in the economic analyses.
3. Results

Fruit Set, Size and Yield

Artificial pollination with ‘Barouni’ pollen significantly increased IFS and FFS in heavy flowering trees in the “on” year (2017). The IFS was 0.87 fruit/panicle in control trees not receiving ‘Barouni’ pollen, while treatments under AP averaged between 1.23 and 1.72 fruit panicle\(^{-1}\) (Table 1). On the other hand, FFS was very low (0.07 fruit panicle\(^{-1}\)) in control trees, but significantly higher in trees exposed to AP (between 0.22 and 0.38 fruit panicle\(^{-1}\)) (Table 1).

The number of ‘Barouni’ pollen applications had an effect on fruit set. Thus, IFS was significantly higher in treatments with two and four pollinations (1.72 and 1.62 fruit panicle\(^{-1}\), respectively), than in trees receiving only one pollination (1.27 fruit/panicle). The highest FFS was observed after four applications, a level of 0.38 fruit panicle\(^{-1}\), significantly higher than FFS obtained after one and two pollen applications (0.22 and 0.24 fruit panicle\(^{-1}\) respectively). The control treatment had only 0.07 fruit panicle\(^{-1}\) (Table 1).

Conversely, in 2018 (“off” year), the number of pollen applications did not increase IFS or FFS, and similar results were obtained in all AP treatments, regardless of the number of ‘Barouni’ pollen applications (IFS was between 0.27 and 0.31 for all AP treatments, while FFS fluctuated between 0.10 and 0.12 fruit panicle\(^{-1}\)). The control treatment showed higher IFS with 0.68 fruit/panicle, but significantly lower FFS (0.06 fruit panicle\(^{-1}\)) (Table 1). Fruit set levels were lower, too, than those measured in “on” trees the year before. It is important to remember that the trees of the control treatment in 2018 were in a different plot 5 km away from AP trees, although under the same management.

The distribution of harvested fruits by size showed a slight inverse trend compared to the yield in 2017—that is, the larger the crop, the greater percentage of small fruits and the higher the increase in the percentage of petite fruits rejected for export due to their insufficient size (Figure 1). It should be noted that the rejected fruit are very small-sized fruit of low commercial value. The treatment with two applications had the highest percentage of large-sized fruits (31.3%), while the lowest proportion of the large-sized fruit was found when only one AP was performed (19.5%). However, in this last treatment, 65% of the fruits were of medium fruit size. The lowest percentage of petite and small-sized fruit was also found in this treatment (Figure 1). The poor harvest of fruits on the tagged shoots of the control treatment did not permit a reliable estimate of the fruit size distribution. The economic analysis of the treatment with four pollinations projected that this treatment generated a value of 64.08 USD tree\(^{-1}\), an amount higher than that obtained with the treatments of one and two pollinations with 41.33 and 43.90 USD tree\(^{-1}\), respectively (Table 2).

Fruit size distribution did not show a clear trend in 2018, as IFS and FFS were similar among treatments of AP. However, in comparison with the previous season (“on” year) fruit size was positively influenced by the low yield in this “off” year (Table 1). Thus, 51.8% of harvested fruits in the treatment with one AP were extra-large and only 4% were of petite size. Only 37.4% of the harvested fruits from the trees receiving four pollinations were also extra-large. In total, 95% the fruit in the treatment with two pollinations were in the large size category and 5% were petite, although it should be noted that was the only treatment with no extra-large fruits (Figure 2). Contrary to what was observed in 2017, increasing the number of AP applications did not improve profits in 2018 (“off” season), because neither yield nor fruit size responded positively to a higher number of pollinations (Table 3).
Table 1. Fruit set, size and estimated yield in monovarietal ‘Manzanillo’ olive orchards with different frequencies of artificial pollination (2017 and 2018).

<table>
<thead>
<tr>
<th>Treatments (No. Pollinations)</th>
<th>2017 (“On”)</th>
<th>2018 (“Off”)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Fruit Set (Fruit Panicle⁻¹)</td>
<td>Final Fruit Set (Fruit Panicle⁻¹)</td>
</tr>
<tr>
<td>0</td>
<td>0.87 c a</td>
<td>0.07 c</td>
</tr>
<tr>
<td>1</td>
<td>1.23 b</td>
<td>0.22 b</td>
</tr>
<tr>
<td>2</td>
<td>1.72 a</td>
<td>0.24 b</td>
</tr>
<tr>
<td>4</td>
<td>1.62 a</td>
<td>0.38 a</td>
</tr>
</tbody>
</table>

* a Values followed by the same letter in each column are not significantly different at \( p \leq 0.05 \).

Figure 1. Olive fruit size distribution according to quality standards: large size (160/180 fruit kg⁻¹), medium size (200/240 fruit kg⁻¹), small size (280/320 fruit kg⁻¹) and rejected (petite size). Adaptation from [39]. (A) one pollination; (B) two pollinations; (C) four pollinations. Trial 2017.

Figure 2. Olive fruit size distribution according to quality standards: extra-large size (130/150), large size (160/180 fruit kg⁻¹), medium size (200/240 fruit kg⁻¹), small size (280/320 fruit kg⁻¹) and rejected (petite size). Adaptation from [39]. (A) one pollination; (B) two pollinations; (C) four pollinations. Trial 2018.
Table 2. Effects of different frequencies of artificial pollination on yield and income in heavy-flowering ‘Manzanillo’ trees. Year 2017.

<table>
<thead>
<tr>
<th>Treatments (No. Pollinations)</th>
<th>Fruit Size</th>
<th>Price (USD kg(^{-1})) (^a)</th>
<th>Yield per Size (kg Tree(^{-1})) (^b)</th>
<th>Yield (kg Tree(^{-1})) (^c)</th>
<th>Pollen Cost (USD Tree(^{-1}))</th>
<th>Income (USD Tree(^{-1})) (^d)</th>
<th>Income (USD ha(^{-1})) (^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Big</td>
<td>-----</td>
<td>-----</td>
<td>3.88</td>
<td>0.00</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.40</td>
<td>6.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>1.40</td>
<td>20.70</td>
<td>31.25</td>
<td>0.90</td>
<td>41.33</td>
<td>5166</td>
</tr>
<tr>
<td></td>
<td>Petite (^f)</td>
<td>0.70</td>
<td>1.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Big</td>
<td>1.40</td>
<td>11.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.40</td>
<td>15.46</td>
<td>35.40</td>
<td>1.80</td>
<td>43.90</td>
<td>5488</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>1.05</td>
<td>6.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petite</td>
<td>0.70</td>
<td>2.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Big</td>
<td>1.40</td>
<td>12.96</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.40</td>
<td>25.47</td>
<td>53.26</td>
<td>3.60</td>
<td>64.08</td>
<td>8010</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>1.05</td>
<td>10.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petite</td>
<td>0.70</td>
<td>4.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) [38]. \(^b\) Based on the proportion harvested for each fruit size category (Figures 1 and 2). \(^c\) Calculated from flowering level, final fruit set and weight. \(^d\) Income = \(\sum\) (yield in each fruit size category \(*\) category price) – application cost. \(^e\) Calculation based on number of trees ha\(^{-1}\). \(^f\) Rejected for exportation (petite size). \(^g\) No data (ND).
Table 3. Effects of different frequencies of artificial pollination on yield and income in light-flowering ‘Manzanillo’ trees. Year 2018.

<table>
<thead>
<tr>
<th>Treatments (No. Pollinations)</th>
<th>Fruit size</th>
<th>Price (USD kg(^{-1})) (^{a})</th>
<th>Yield per Size (kg Tree(^{-1})) (^{b})</th>
<th>Yield (kg Tree(^{-1})) (^{c})</th>
<th>Pollen Cost (USD Tree(^{-1}))</th>
<th>Income (USD Tree(^{-1})) (^{d})</th>
<th>Income (USD ha(^{-1})) (^{e})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>---</td>
<td>ND (^{f})</td>
<td>ND</td>
<td>4.83</td>
<td>0.00</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>Extra large</td>
<td>1.30</td>
<td>9.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Big</td>
<td>1.40</td>
<td>1.96</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.40</td>
<td>1.68</td>
<td>11.41</td>
<td>0.90</td>
<td>16.37</td>
<td>2046</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>1.05</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petite (^{f})</td>
<td>0.70</td>
<td>0.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Extra large</td>
<td>1.30</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Big</td>
<td>1.40</td>
<td>9.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.40</td>
<td>0.00</td>
<td>10.12</td>
<td>1.80</td>
<td>13.81</td>
<td>1726</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>1.05</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petite</td>
<td>0.70</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Extra large</td>
<td>1.30</td>
<td>3.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Big</td>
<td>1.40</td>
<td>4.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.40</td>
<td>0.00</td>
<td>8.48</td>
<td>3.60</td>
<td>11.22</td>
<td>1403</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>1.05</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petite</td>
<td>0.70</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) [38]. \(^{b}\) Based on the proportion harvested for each fruit size category (Figures 1 and 2). \(^{c}\) Calculated from flowering level, final fruit set and weight. \(^{d}\) Income = \(\sum\) (yield in each fruit size category \(\times\) category price) – application cost. \(^{e}\) Income = \(\sum\) (yield in each fruit size category \(\times\) category price) – application cost. \(^{f}\) Calculation based on number of trees ha\(^{-1}\). \(^{g}\) Rejected for exportation (petite size). \(^{h}\) No data (ND).
4. Discussion

AP is an increasingly widespread practice in cross-pollinated crops [40]. Due to the methods of implementation, its use seems easier in anemophilous species in which the mechanical application (through the use of dusters) reproduces, with certain reliability, the natural pollination vector (the wind). Previous pollen collection is also simpler and cheaper in anemophilous crops and pollen quality measurement and its preservation in storage is commonly easier and less expensive than in entomophilous crops [15]. AP has proven effective in olives to replace free pollination, improving yields in monovarietal blocks of self-incompatible genotypes [14], especially where arid conditions reduce the effectiveness of self-pollination [16].

In plantations that include pollinizers, AP has the advantage of not assigning an important area to varieties of lower commercial value. Previous authors suggest this percentage to be between 20% and 25% [41]. However, in order to wisely recommend AP instead of pollinizer use, the first assessment to be made is whether the expenses represented by AP are offset by the increase in yield and fruit quality. Thus, in this experiment, we first determined the economic benefits of different AP treatments, and then, once the profitability of AP was established, a comparison with the incomes obtained in orchards including pollinizers in their design was discussed.

In 2017, ‘Manzanillo’ olive trees showed increases in fruit set in response to AP, as found by Grijalva-Contreras et al. [17,42] and before by Sibbett et al. [14] in similar agroenvironmental conditions. In this season, we found a higher level of initial and final set with an increase in the number of pollinations, matching the results reported by Sibbett et al. [14], applying the same number of applications at a rate of 75 g ha⁻¹ of ‘Sevillano’ undiluted pollen at the same phenological stages. ‘Sevillano’ pollen induced a fruit set similar to ‘Barouni’ pollen on ‘Manzanillo’, as demonstrated in a recent research carried out in the experimental area [9]. Similar results have been obtained in some European countries. Gianni and Vania [13] have shown an improvement in fruit set between 10% and 30% in ‘Leccino’ when ‘Casaliva’ pollen was applied twice (at between 50% and 95% of flowering) at a dose of 2 g per tree. In ‘Picual’, the main olive oil cultivar in Spain, supplementary artificial pollination with pollen of different cultivars was also positive, increasing the final fruit set greatly (between 67% and 550%, depending if the plot was irrigated or rainfed, respectively), but only with respect to self-pollination, failing to increase the fruit set obtained through open pollination in mixed orchards [43]. With hazelnuts, fruit set increased by a percentage of 37% when a mix of cross-pollen from different tree donors was applied to female flowers under adverse environmental conditions [28]. With pistachios, fruit set was improved after AP with respect to open pollination [26]. In another experiment, a 16% greater yield was obtained in treated trees compared to control trees in plantations in the San Joaquin Valley (CA) after electrostatic AP, a novel technique that aims to enhance pollen adhesion to female pistachio flowers [44]. Finally, in date palms, sprinkled pollen in liquid suspension applied directly to the inflorescence increased fruit set by 40% compared to open-pollinated trees [45].

AP has therefore been proven successful in increasing fruit set and/or yield in a variety of wind-pollinated crops. The next step in this process is to determine the optimal dose of cross-pollen applied. In our experimentation, the most profitable treatment was four pollinations. This treatment represents an almost constant supply of cross-pollen during the entire flowering period, in a similar manner to orchards that include pollinizers [46]. Under this condition of free cross-pollination, without restrictions, the highest levels of fruit set are frequently obtained in multivarietal plantations [47,48]. However, the designs of multivarietal plantations impose a considerable area of cultivation assigned to varieties of lower commercial value than that of the main variety.

An additional aspect to consider in the AP is the choice of application timing, taking into consideration that the first flowers that open are of better quality [49] and have greater potential for fruit set. In all AP treatments, we supplemented pollen in the phenological stage of full flowering, seeking to reach the largest number of open flowers. The last two pollen applications in the four-application treatment covered the end of the flowering period, when a remnant of hermaphrodite flowers open and can be fertilized [49], but whose fruit set is, perhaps, less probable if the initial fruit set level has
been satisfactory. Our results suggest, however, a substantial effect of late AP applications in 2017 (“on” year), adding large extra profits (roughly 2000 USD ha$^{-1}$ more); see Table 2). No extra profits were observed in light-flowering trees in 2018, when only one application of cross-pollen was enough to increase profits to the maximum level (Table 3).

The yield enhancement obtained in 2017 in heavily flowering trees is slightly below the yield increase reported by Grijalva-Contreras et al. [17], but slightly higher than the increase obtained in another experiment where ‘Manzanillo’ trees were artificially cross-pollinated in combination with a biostimulant (a mix of vitamins, mineral elements and phytohormones such as auxins, gibberellins and cytokinins) [42]. By combining these two applications, the authors obtained lower yields but higher fruit sizes compared to those reported in this work.

In 2017, fruit distribution by size was characterized by a higher percentage of large fruits in the AP treatments with lower fruit sets, with this circumstance being explained by less competition between fruits within the same shoots [50,51]. In other words, the higher the number of fruits per panicle, the greater the fruit competition for assimilates and, consequently, the smaller the fruit size, a trend that was reflected in their distribution by size, where a higher yield generated a greater percentage of petite fruits and a lower proportion of large fruits.

In 2018 (“off” year), FFS levels were low and no differences were found among AP treatments, although significant differences were present between AP treatments and control trees that did not receive supplementary pollination. Although the flowers produced in “off” trees are of better quality and more likely to set fruit [52], the positive effects of repeated applications of cross-pollen seemed diluted due to the short flowering period. Logically, in the “off” year (2018), trees had a greater proportion of fruit in higher categories by size and a lower percentage of small fruit. In other anemophilous species such as dates or pistachios, fruit weight improves substantially when pollen is artificially supplemented, especially with pollen from specific cultivars [25,26]. In dates and pistachios, the phenomena of xenia and metaxenia (pollen source effects on seeds and fruit, respectively), have been reported [53]. In the olive tree, there is no evidence of these phenomena, although the size of the seed clearly affects the size of the fruit [54,55]. The results suggest that scattered bloom in “on” trees requires a higher number of pollen applications, while the low number of flowers formed in “off” trees are well-covered by only one pollen application due to the shorter flowering period.

Finally, once the profitability of AP in olive orchards in Mexico is proven, a comparison of the profits obtained under AP versus pollination designs can be performed. In a recent experiment, a benefit of 5983 USD ha$^{-1}$ was obtained in the most profitable pollination design for Caborca (Mexico) ‘Manzanillo’ olive orchards (1:1 ratio of ‘Barouni’:‘Manzanillo’) [56]. The benefits obtained under the best AP strategy (four applications of ‘Barouni’ pollen) were much higher (8010 USD ha$^{-1}$) in our experiments. However, our results require confirmation in other non-traditional olive producing countries.

5. Conclusions

The results presented in this study show that AP improves fruit set in ‘Manzanillo’ monovarietal olive groves, confirming the potential of AP in these conditions. The results also indicate that a greater number of applications, maintaining a constant exposure of ‘Manzanillo’ flowers to ‘Barouni’ pollen during the entire flowering period, resulted in greater economic benefits despite its higher cost. Nonetheless, it should be noted that an improvement with an increasing number of applications was only observed during the “on” year. The increase in fruit set negatively affected the average size of the fruit and its commercial distribution by size. However, this did not represent a significant decrease in the value of the crop. Our results support the use of AP in ‘Manzanillo’ monovarietal olive orchards in Mexico.

**Author Contributions:** A.S.-E. did most of the fieldwork and formal analyses and contributed to the writing of the original draft. J.C. designed the experiments, supervised the work, and edited and translated the submitted manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.
Conflicts of Interest: The authors declare no conflict of interest.

References
19. Johansen, C. Artificial pollination of apples with bee-collected pollen. J. Econ. Entomol. 1956, 49, 825–828. [CrossRef]
Agronomy 2020, 652


45. Iqbal, M. Effect of different pollination techniques on fruit set, pomological characters and yield of Dhakki date palm (Phoenix dactylifera L.) in Dera Ismail Khan, KP. J. Agric. 2010, 26, 515–551.


51. Suárez, M.P.; Fernández-Escobar, R.; Rallo, L. Competition among fruits in olive II. Influence of inflorescence or fruit thinning and cross-pollination on fruit set components and crop efficiency. *Acta Hortic.* 1984, 149, 131–144. [CrossRef]


55. Farinelli, D.; Pierrantozzi, P.; Palese, A.M. Pollenizer and cultivar influence seed number and fruit characteristics in *Olea europaea* L. *HortScience* 2012, 47, 1430–1437. [CrossRef]


© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).