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Research Paper

‘Arbequina’ olive is self-incompatible

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A R T I C L E I N F O

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A B S T R A C T

‘Arbequina’ is the olive cultivar preferred by growers for the design of the trendy high-density orchards due to its low vigor, high yield, and extraordinary oil quality. Although, ‘Arbequina’ is considered by many authors as self-compatible, increasing evidence questions its full self-compatibility. Our results clearly illustrate that, contrary to earlier assumptions, ‘Arbequina’ olive behaves as a self-incompatible variety. Self-pollen rejection in the style, low levels of self-fertilization and decreased initial and final fruit set after self-pollination in comparison to cross-pollination treatments clearly indicate that ’Arbequina’ is self-incompatible. Furthermore, even seed and fruit size were depressed in response to self-pollination, despite the lighter crop produced in self-pollinated shoots. This was observed in both ‘on’ and ‘off’ ‘Arbequina’ trees. Both pollinizers tested, ‘Manzanillo’ and ‘Picual’, matched results obtained under free open-pollination and significantly increased the yield obtained under self-pollination. Our results suggest the convenience of establishing pollinizers for large, high-density ’Arbequina’ orchards, especially in new areas of olive cultivation, where wind pollination may be not enough to ensure beneficial cross pollen flow.

1. Introduction

Olive is a wind-pollinated, partially self-incompatible fruit crop (Arbeiter et al., 2014; Cuevas et al., 2001; Griggs et al., 1975; Taslimpour and Aslmoshtaghi, 2013). Despite the partially self-incompatible condition of the species, some olive varieties exhibit a strong self-incompatible response (Guerin and Sedgley, 2007; Koubouris et al., 2014; Zhu et al., 2013) and therefore require pollination designs that must include compatible pollinizers in adequate number and places (Cuevas and Polito, 2004; Shemer et al., 2014). On the contrary, ‘Arbequina’ cultivar has been considered by many as fully self-compatible (Serrano et al., 2012), especially due to its high yields in high density orchards designed without pollinizers (Rovira and Tous, 2002). Nonetheless, given the extraordinary and unsuspected importance of free wind-pollination in traditional olive producing countries (Pinillos et al., 2012; Pinillos and Cuevas, 2009), it cannot be ruled out that many ‘Arbequina’ fruits are actually fathered by cross-pollen as proven previously by Díaz et al. (2006) and recently by Marchese et al. (2016) and Wang et al. (2017).

Self-incompatibility reaction in olive is characterized by a reduction in pollen germination (not always significant) and a clear delay in pollen tube growth in the style and consequently by late and reduced levels of self-fertilization (Cuevas and Polito, 1997; Saumitou-Laprade et al., 2017; Vuletin Selak et al., 2014). In this context, the duration of ovule longevity seems critical. Ephemeral flowers with short-lived ovules are strongly dependent on fast growing pollen tubes, such as those formed by cross-compatible cultivars (Vuletin Selak et al., 2014). This is especially true for heavy flowering ‘on’ trees, that present lower flower quality and a shorter effective pollination period than those measured in light flowering ‘off’ trees. An improvement in flower quality has been also observed in ‘off’ trees for pollen viability and germinability (Mazzeo et al., 2014).

To elucidate the compatibility behavior of ‘Arbequina’, we designed an experiment analyzing its response to different pollination treatments, including pollen-pistil interaction, fruit production, and seed and fruit characteristics. We compared ‘Arbequina’ response to self-pollination with respect to open- and cross-pollination in ‘on’ as well as in ‘off’ trees, and relate the response of ‘Arbequina’ to the differential flower quality and fertility observed in heavy versus light flowering trees.

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2. Material and methods

2.1. Plant material and orchard location

The experiment was carried out in 2015 in a solid monovarietal 20-years-old orchard of 'Arbequina', sited at the facilities of the Institute of Agricultural and Fishery Research and Training (IFAPA), in La Cañada de San Urbano, Almería, Spain. According to Papadakis' classification, the climate of the area is of the type Mediterranean semiarid-arid (Gómez-Zotano et al., 2015). Rain episodes at the experimental site are scarce with an average annual accumulate of 250 mm, mostly during autumn and winter. Average mean daily temperatures oscillate between 15.5 and 19.0 °C with maximum means ranging from 20.5 to 24.8 °C and minimum means from 10.6 to 14.0 °C.

Single trunk olive trees vase-trained at a planting distance of 6 × 8 m were used for the experiments. Orchard management includes drip irrigation and non-tillage. Weeds are controlled with herbicides. In this orchard, we selected four trees with a high level of flowering ('on' trees) and four trees with a reduced number of inflorescences ('off' trees) for the experiments. On these trees, flowering level, fertility and quality were estimated. Selected 'on' trees averaged a level of 8.75, while 'off' trees reached a level of 3.75, in a hedonic scale between 0 (no flowering) and 10 (very high level of flowering). The average number of flowers per panicle was calculated sampling 20 panicles per tree (Table 1).

Flower fertility was estimated by the percentage of inflorescences with at least one hermaphrodite flower (and hence with capacity to set fruit), whereas the quality of the flower was considered related to its size estimated by its dry weight (Cuevas et al., 1994a). Flower dry weight was averaged from 20 hermaphrodite flowers per tree sampled from inflorescences situated at observer's height and at the four cardinal points. All sampled flowers occupied the apical position of the panicle ("king" flower) (Cuevas and Polito, 2004).

2.2. Experimental design and treatments

The trial was designed as a nested experiment in which the level of flowering, heavy ('on) and light ("off), were the two levels of the first factor, while the four pollination treatments (self-, open-, and cross-pollination using fresh pollen grains from 'Picual" and cross-pollination using fresh pollen grains from "Manzanillo") acted as the levels of the second factor. All pollination treatments were applied on a sample of eight 1-year-old shoots on each of the same four 'on' trees and on each of the same four 'off' trees. The reproductive shoots per tree and pollination treatment functioned as subsamples within each replication (trees). These shoots were located at observer's height around the canopy. The number of panicles was adjusted by hand to 14 per shoot in 'on' trees, and to 6 per shoot in 'off' trees for homogeneity purposes.

Self-pollination was achieved by covering shoots before flowering with hand-made bags of tissue paper. Self-pollen deposition was favored by frequent shaking of the shoots. The bags were not removed until the flowering season had ended. Open-pollinated shoots were left uncovered and received wind-dispersed pollen from nearby trees. 'Picual', 'Arbosana' and 'Lechín de Granada' olive trees were recognized near the orchard in a range of 100 m. Cross-pollination treatments were achieved by applying fresh pollen to the stigmas of open flowers of each shoot using a fine paintbrush. Hand cross-pollination commenced at the beginning of bloom and was repeated twice on alternate days (Cuevas and Polito, 1997). Pollen grains used for cross-pollination were previously harvested from a nearby olive orchard following the procedures described in Cuevas and Pinillos (2006). Pollen viability was ascertained before use by the fluorochromatic staining test proposed by Heslop-Harrison and Heslop-Harrison (1970), slightly modified by Pinillos and Cuevas (2008). Pollen viability of pollinizers was 57.2 and 47.8% for 'Manzanillo' and 'Picual', respectively. Cross-pollinated flowers remained bagged before and after hand pollinations until stigma senescence took place.

Table 1
Flower number per panicle, fertility and size in 'on' and 'off' 'Arbequina' trees.

Crop Load	Flowers per panicle	Fertile Panicles (%)	Hermaphrodite flowers (%)	Flower dry weight (mg)
on	24.1 a[a]	99.1 a	77.6 a	2.8 b
off	15.4 b	100.0 a	83.6 a	3.4 a

[a] Values followed by the same letter in each column are not significantly different at $p \leq 0.05$.

2.3. Pollen-pistil interaction

Pollen-pistil interaction was studied in each pollination treatment on a sample of 20 flowers collected 4, 6 and 8 days after pollination (60 flowers in total). Pollination was always performed on flower anthesis. Anthesis date was assured by removing open flowers on a given day, and closed flowers the next day, so all remaining flowers opened between one given day and the next one. Pollination treatments were applied as explained in 2.2. Sampled flowers were excised from the shoots 4, 6 and 8 days after pollination (dap) and fixed in FAE (formaline: glacial acetic acid: ethanol 70%, in a ratio 1:2:17 v/v) until observations. Fixed flowers were processed as explained in Cuevas et al. (1994b) and stained using aniline blue for observations under fluorescence microscopy (Martin, 1959) in a Nikon Labophot epifluorescence microscope.

Pollen adhesion, germination, pollen tube growth in the style, fertilization levels and date were determined in each flower and the results averaged for each pollination treatment and date. The number of pollen grains adhered to the stigmas of the flowers was estimated by counting the number of pollen grains in three small areas of the stigma, and then calculated considering the common size of the olive flower stigma (Griggs et al., 1975). Pollen germination was expressed as the ratio between pollen grains adhered and those germinated, i.e. forming a pollen tube and penetrating the stigma. Pollen tube growth in the style was labeled as null (no pollen tubes seen in the style), scarce (fewer than five pollen tube tubes present in the style), moderate (from 5 to 25 pollen tubes observed) and massive (over 25 pollen tubes per flower). An ovule was considered fertilized if a pollen tube was present at the micropyle of it or if the ovule had become a developing seed, i.e. the ovule had grown to more than three times its initial size and the other three ovules of the olive pistil were senescent (Cuevas et al., 1994b; Seifi et al., 2011).

2.4. Initial and final fruit set, fruit and seed weights and self-Incompatibility index

Initial (15 days after pollination; dap) (IFS) and final fruit set (45 dap) (FFS) were determined on tagged shoots as the number of fruits per panicle. Shotberries were not counted. Self-incompatibility index was then calculated for both periods (IISI and FISI) as the ratio between fruit set under self- and open- and cross-pollination (Zapata and Arroyo, 1978). Self-incompatibility index usually varies between 1 (full self-compatibility) and 0 (strong self-incompatibility). Near fruit maturity, in October 2015, all present fruits were harvested and weighed in the lab, where the stones were removed from the pulp and the seeds contained extracted. The number of viable seeds and their fresh weight were then recorded. Initial and final fruit set, self-incompatibility indexes, and fruit and seed weight were compared by analyses of variance. Tukey's test was used for the separation of the means. Chi-square analyses were performed for the comparison of fertilization levels and of the number of seeds per fruit. Infostat 2017 version software was used for these analyses.