

M. Librada Alcaraz and
J. Ignacio Hormaza

Instituto de Hortofruticultura Subtropical y Mediterránea
“La Mayora,”
Universidad de Málaga-Consujo Superior de
Investigaciones Científicas (IHSM-UMA-CSIC),
Estación Experimental “La Mayora,”
29760 - Algarrobo-Costa, Málaga, Spain

Avocado Pollination and Fruit Set – A Perspective from Spain

Introduction

The avocado (*Persea americana* Mill.) is an evergreen subtropical fruit tree native to Central America and Mexico where it was domesticated and cultivated since ancient times (Galindo-Tovar et al., 2008; Chen et al., 2009). It is a member of the Lauraceae, a mostly subtropical or tropical family included within the order Laurales in the evolutionary early-divergent angiosperm clade Magnoliid. Spain is the only European country with a significant commercial production of avocados, confined to the Andalusian Mediterranean coast, mainly in the provinces of Malaga and Granada.

The avocado flower is bisexual having both functional male and female organs although separated in time through a synchronous protogynous dichogamous breeding system

that prevents self-pollination and promotes outcrossing. Each perfect flower opens twice, the first functionally as a female flower with a white receptive stigma; then the flower closes and the following day the flower reopens functionally as a male flower, with the stigmas no longer receptive, and dehisced anthers (Davenport, 1986). The different avocado cultivars are classified in two groups (A or B) based upon their flowering behaviour (Nirody, 1922). In the type A cultivars, the flowers open in the morning in the female stage, close at midday and reopen the afternoon of the following day in the male stage. In the type B cultivars, the flowers open in the afternoon in the female stage, close in the evening and reopen the following morning in the male stage (Stout, 1923). However, this floral behaviour is only observed under optimum climatic conditions because the cycle may be disrupted due to its extreme sensitivity to environmental conditions particularly to cool temperatures (Stout, 1923; Lesley and Bringhurst, 1951; Bergh, 1969, 1974; Sedgley and Annells, 1981).

One of the main limitations in avocado production worldwide is the massive abscission of flowers and developing fruits primarily in the first two months following flowering. This results in less than 1% of the fruits remaining on the trees at harvest (Cameron et al., 1952; Sedgley, 1980; Lahav and Zamet, 1999; Garner et al., 2008; Garner and Lovatt, 2008). An excess of flower production and a low fruit-to-flower ratio is a common phenomenon in plants with hermaphrodite flowers (Stephenson, 1981; Sutherland and Delph, 1984; Sutherland, 1986), although this situation is extreme in avocado since the average fruit set obtained in hermaphrodite plants has been estimated around 40% (Sutherland and Delph, 1984). Studies of immature fruit abscission in different species suggest that the low flower to fruit ratio is not necessarily only the result of inadequate pollination and that several additional factors could be involved.

Here we summarize our recent results on avocado flowering and fruit set in Spain. We have to stress the fact that the results obtained represent those under the environmental conditions in Southern Spain and, consequently,

they might not be directly applicable to different growing conditions. Data obtained at the E.E. la Mayora (36° 45' N), where the experiments have been conducted, in the last 45 years show an annual average of the mean temperatures of 19.4°C. The average high temperature in the warmest month (August) is 29.4°C, and the average low temperature in the coldest month (January) is 9.3°C. The average annual rainfall is 435 mm. Temperatures in April (when 'Hass' avocado flowering takes place) during the last four years have ranged from 9°C to 27°C with an average of 16°C. The relative humidity ranged from 45 to 94% with an average of 72%.

Thus, with the purpose of understanding the reproductive factors that limit avocado production under Spanish growing conditions we analyzed the progamic phase, which is the phase from pollination to fertilization, in 'Hass' and its implications in final fruit set. In order to take into account the biennial bearing of avocado these studies were carried out during two consecutive years.

Pollination as a limiting factor

To estimate the effect of pollination on fruit set, we characterized the flower and fruit drop pattern in two populations of flowers (Fig. 1): one was hand-pollinated and the other was left to open pollination.

The initial flower drop was followed by a high fruit drop (Fig. 2) during the two months following flowering (under our growing conditions May and June). Sedgley (1987) reported that two weeks after flowering there is a preferential drop of unpollinated flowers. Tomer and Gazit (1979) noted that three or four weeks after initial fruit set there is a drop of small fruits which was attributed to the lack of endosperm and embryo in cultivars like 'Fuerte' and 'Ettinger'. Similar results where the heaviest flower and fruit abscission takes place in spring and summer have been reported for different cultivars in different countries (Garner and Lovatt, 2008 and references therein).

Our results show that a similar drop pattern occurred in hand-pollinated flowers in the high (on) and low (off) bearing years suggesting that the main difference in production



Figure 1. Flowers labeled during the flowering season showing the date of anthesis and position into the inflorescence to determine the flower/fruit pattern

between both years is due to the higher production of flowers during the high bearing year. Thus, these results corroborate previous results suggesting that the percentage of abscission does not depend on flower intensity (Garner and Lovatt, 2008; Garner et al., 2008).

The percentage of fruit set obtained in hand-pollinated flowers was fifteen times higher than in flowers left to open-pollination suggesting that a higher fruit set could be obtained by increasing the supply of pollen to the flower during the female phase. This indicates that the lack of pollination could be one of the limiting factors in commercial avocado



Figure 2. Fructification process. (a) Inflorescence with flowers at anthesis. (b) Honeybee collecting pollen from male flowers. (c) Female flower being pollinated by a honeybee. (d) Inflorescence at the end of the flowering season after drop of a high number of flowers. (e) Fruit development three weeks after pollination. (f) Fruit reaching the maturity stage.

production. However, we also found an early abscission pattern in both hand- and open-pollinated flowers, suggesting that other factors besides lack of pollination contribute to the massive drop of avocado flowers and fruitlets.

Effect of distance to the pollinizers on yield

Traditionally it has been recommended to interplant complementary A and B avocado cultivars to increase yield. A positive effect of interplanting complementary cultivars in close proximity on yield in avocado has been found in Israel (Degani et al., 1989; 1997) whereas in California, the results have ranged from some correlation (Bergh and Gustafson, 1958, 1966; Bergh and Garber, 1964; Bergh, 1966, 1968; Vrecenas-Gadus and Ellstrand, 1985; Kobayashi et al., 2000), to the absence of correlation (Garner et al., 2008). Similarly, in South Africa, no clear effects of distance to the pollen source on fruit yield have been obtained (Robbertse et al., 1996; Johannsmeier and Morudu, 1999). Likewise, the relative impact of outcrossing on final yield in 'Hass' is a subject of controversy, probably because the studies have been performed in avocado growing areas with different environmental conditions (Garner et al., 2008). Moreover, satisfactory yields occur in orchards composed of a single variety probably due to both self-pollination (in the male stage when the stigma is receptive) and close-pollination (pollination during an overlap of male and female flowers in the same inflorescence or tree) (Davenport, 1986; Ish-Am, 1999).

Considering the contradictory results in different avocado growing areas, we addressed this topic under the environmental conditions of the Southern Spanish coast determining the outcrossing rate at harvest time on several 'Hass' trees located at different distances from a solid 'Fuerte' plot during two consecutive years. We also analyzed the effect of distance to the pollinizer trees on yield using data available from 12 consecutive years in the same orchard. This is especially important in economic terms because the price of the fruits produced by the pollinizers is usually significantly lower than those derived from 'Hass' and, therefore, the cost of using a space of the orchard to interplant pollinizer trees

could be higher than the higher input derived from the possible increasing yields obtained by outcrossing (Schnell et al., 2009). In this work, when we refer to self-pollination we include both self-pollination and close-pollination (Ish-Am, 1999) since in both cases the resulting fruits are indistinguishable using molecular markers.

Under our growing conditions, we found a decrease in the percentage of outcrossing with increasing distance from the pollinizers similar to previously described results (Vercenar-Gadus and Ellstrand, 1985; Degani et al., 1989; 1997; Kobayashi et al., 2000; Garner et al., 2008), although no significant correlation between outcrossing rate and yield was established in both years of study. Likewise, no significant effect of distance between complementary cultivars on yield was observed during the twelve years analyzed.

We also examined the outcrossing rate between 'Hass' and 'Fuerte' in a situation where the two varieties were interplanted. We observed that the level of self-fertilization was higher in 'Fuerte' than in 'Hass'. This could be due to the fact that the two varieties only overlap in flowering for approximately three weeks (Alcaraz and Hormaza, 2009): at the beginning of the 'Fuerte' flowering season no pollen from 'Hass' flowers is available and the reverse is the case for 'Hass' at the end of its flowering season. We also observed abnormalities in the normal cycle of 'Fuerte' flowers that could extend the overlap between male and female flowers increasing the chances for self-pollination (Alcaraz and Hormaza, 2009).

The effect of the distance between cultivars on yield has been mainly explained in terms of a selective drop of fruits resulting from self-fertilization (Degani et al., 1989; 1990; 1997). Thus, in spite of the high percentage of self-pollination reported under different environmental conditions (Davenport, 1989; Davenport et al., 1994), most of the final fruits are the result of outcrossing (Vercenar-Gadus and Ellstrand, 1985; Degani et al., 1986, 1989, 1997, 2003; Goldring et al., 1987; Robbertse et al., 1997; Chen et al., 2007; Borrone et al., 2008). In order to study this topic under our environmental conditions, after the flowering season, several fruits were tagged based on the week of fertilization (at the end, mid and start of the 'Hass' flowering season). A

polyethylene net was placed on the ground under each 'Hass' tree to collect all the falling fruitlets from June to commercial harvest in March the following year. Our results show a high drop of fruits derived from self-fertilization from June to August. However, if we take into account the time of fertilization most of those fruits are fertilized at the end of the 'Hass' flowering season, suggesting that this selective fruit drop could be mainly due to resource competition rather than to genetic selection among developing embryos. Since under our growing conditions, no 'Fuerte' pollen is available at the end of the 'Hass' flowering season, the fruits fertilized at the end of the 'Hass' flowering season are the result of self-pollination. These fruits have to compete for nutritive resources with those derived from flowers pollinated at the beginning of the 'Hass' flowering season when 'Fuerte' pollen was available. Consequently, the results obtained indicate that outcrossing is probably not the main limiting factor in 'Hass' yield under the growing conditions of Southern Spain and that competition by available resources could be the main reason behind fruit drop during the months following the flowering season. This competition occurs not only among developing fruits but also with the new vegetative flush that starts at the end of the flowering period.

Looking for alternative pollinizers

Due to the asynchrony between the flowering periods of 'Hass' and 'Fuerte', we decided to study additional putative pollinizers for the second half of the 'Hass' flowering period. With this objective, the flowering period was studied in 27 avocado genotypes during two consecutive years. The blooming period ranged from 18 to 50 days and full bloom varied from 1 to 2 weeks in most genotypes. Full bloom for most genotypes occurred during April, showing good overlapping with 'Hass'.

Since a good pollinizer must present not only a good overlap in the flowering season but also in sexual stages with the pollinated cultivar, 'Fuerte' and 10 additional 'Hass-like' genotypes that present sufficient overlapping in the blooming period with 'Hass' were studied. We determined daily the

stages of male and female overlapping every 2 hours.

Under our environmental conditions it is common to observe flowers in different sexual stages at the same time both among trees of the same genotype and within the same tree. This overlapping could be considered as a “bet-hedging” strategy to ensure fertilization when the opportunities for outcrossing are limited and may help to explain fruit production in single cultivar blocks (Davenport, 1989; Davenport et al., 1994). The floral behaviour also showed a wide variation during the blooming season; thus, at the beginning of the blooming season, when temperatures are cooler, the floral cycle was longer than the cycle observed at the end of flowering and, in this period, overlapping between the male and female stages could be observed during four hours.

On the other hand, under normal conditions in the blooming season, while type A genotypes showed a similar behaviour to that described for the first time by Nirody (1922), the floral cycle of type B genotypes deviated from the normal cycle. Thus, flowers opened in the female stage in the evening, later than previously described in other avocado growing regions; instead of closing overnight, most of them remained open briefly the following morning; they closed during the day and opened again the following day in the male stage; consequently, it is frequent to observe an overlapping between the female flowers closing the second day of the cycle and the male flowers opening in the morning of the third day of the cycle.

The floral behaviour was mainly altered in days characterized by lower temperatures. ‘Hass’ flowers showed a one day delay in the cycle, and the flowers opened in the male stage at the third day of the flower cycle. The type B genotypes showed higher sensitivity to low temperatures as had been reported previously (Lesley and Bringhurst, 1951; Sedgley, 1977; Sedgley and Annell, 1981; Sedgley and Grant, 1983); thus, under those conditions, flowers opened in the female stage in the evening and, some of them, remained open during the morning and reopened the fourth day in the morning as male flowers.

Results indicate that taking into account the flowering phenology and the overlapping in sexual stages, ‘Marvel’

(BL516) and 'Nobel' (BL667) could be an interesting alternative as pollinizers for 'Hass' in Southeastern Spain. Hand-pollination experiments also showed that a significantly higher fruit set after pollination with 'Marvel' and 'Nobel' compared to 'Fuerte' (Alcaraz and Hormaza, 2009). Different factors such as differences in pollen vigour or differential pollen tube growth during the progamic phase and/or differential embryo abortion in the postzygotic level could be responsible for these differences.

Effect of environmental conditions on the progamic phase

Environmental conditions not only affect flowering behavior but also the progamic phase, from pollination to fertilization. The effective pollination period (EPP) was defined by Williams (1970) as the period which a flower maintains its capacity to be transformed into fruit. This period is conditioned by three events: stigmatic receptivity, pollen tube kinetics and ovule longevity (review in Sanzol and Herrero, 2001). Beside these requirements, a minimum of 20 pollen grains on the stigma is required for an efficient fertilization in avocado (Ish-Am and Eisikowitch, 1995).

In spite of the importance of the length of stigmatic receptivity on the period of effective pollination, little is known about the effect of environmental conditions on EPP in avocado. Several works have reported that stigmas are no longer receptive in flowers opening in the male stage although sometimes pollen grains can germinate but the pollen tubes do not reach the ovule (Sedgley, 1977; Shoval, 1987). However, others have reported that fertilization can occur in this situation (Davenport, 1989, 1991, 1999; Davenport et al., 1994). Due to these differing results, we decided to study the effect of different temperatures and relative humidities on stigmatic receptivity and pollen tube growth through the style. Thus, the effect of four temperatures (15, 20, 25 and 30°C) and three relative humidities (50, 75 and 95%), that are representative of the variation observed under our growing conditions during the 'Hass' flowering season, on stig-

matic receptivity were analyzed in approximately 60 flowers per treatment maintained in wet florist's foam. Stigmatic receptivity was also evaluated under field conditions. The flowers in the female stage were hand-pollinated using pollen from 'Fuerte' and flowers in the male stage using pollen from 'Hass'. The flowers for each treatment were collected periodically over a 3 day period after pollination, fixed and processed to be visualized in squash preparations with 0.1 aniline blue in 0.1N K_3PO_4 under an optical microscope with UV epifluorescence. In all these flowers the minimum number of pollen grains deposited on the stigma was higher than 20. Differences were observed on the adequate conditions to obtain the maximum pollen adhesion and germination in both male and female flower stages. The maximum pollen adhesion and germination was observed at 20 and 25°C and high RH in both male and female stages. These results were similar to those described in different avocado cultivars (Sahar and Spiegel-Roy, 1984; Loupassaki et al., 1997) and to the conditions required for good fruit set (Sedgley, 1987; Sedgley and Annells, 1981). Moreover, we found that at temperatures higher than 25°C, pollen germination decreased mainly in the male stage probably due to rapid stigma degeneration. High relative humidity can prevent the stigma from desiccation during male stage, allowing pollen germination under this condition.

Although a large number of pollen tubes is recorded at the stigma, the number of growing pollen tubes decreases along the style (Tomer and Gottreich, 1975; Sedgley, 1979) and, finally, only one reaches the ovary and fertilizes the ovule (Fig. 3). As in other species (reviewed in Hedhly et al., 2009), avocado pollen tube growth through the style is highly dependent on temperature (Sedgley, 1977; Sedgley and Annells, 1981; Sedgley and Grant, 1983). The pollen tube journey along the style is shortened by increasing temperatures. We observed that in flowers maintained at 30°C the pollen tubes reach the ovary 4 hours after pollination and 8 hours in those maintained at 20°C although in both conditions the pollen tubes were observed near the ovule 24 hours after pollination. However, at lower temperatures (10°C), two days after pollination no pollen tubes were observed penetrating

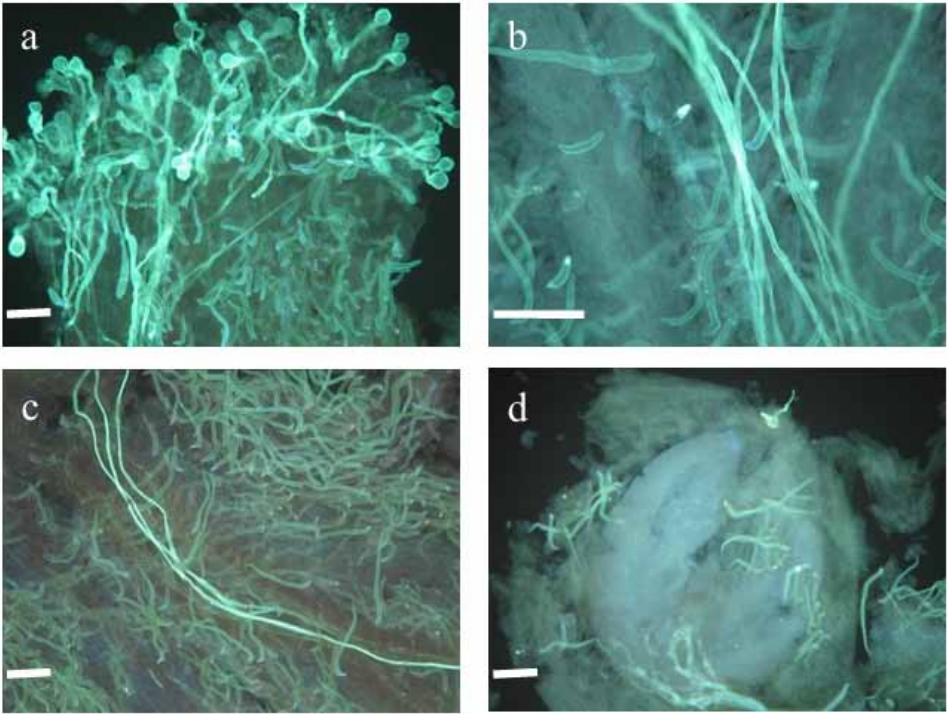


Figure 3. Progametic phase. (a) Pollen germination on the stigma. (b) Pollen tube growth along the transmitting tissue at the upper part of style. (c) Reduction of the number of pollen tubes at the base of style and a single pollen tube entering the ovary. (d) Pollen tube reaching the ovule. Bars 100µm.

the ovule. This observation agrees with the low fruit set obtained under cold conditions in avocado (Sedgley, 1977; Sedgley and Annells, 1981; Sedgley and Grant, 1983).

Furthermore, previous work has described a negative effect of warmer temperatures on ovule viability during the flowering season in other fruit trees (Burgos and Egea, 1993; Stösser and Anvari, 1982; Hedhly et al., 2003). In this experiment we also observed that high temperatures shorten the time of ovule viability although this does not seem to be a limiting factor in those flowers pollinated in the female stage.

Thus, besides adequate pollination, appropriate environmental conditions are also needed for an adequate fertilization. But, while fertilization is a requirement for fruit set, it does not appear to be sufficient for a flower develop into a fruit.

Nutritive reserves

In avocado, no morphological differences are apparent between the flowers that drop and those that will be retained in the trees (Sedgley, 1980). In this species, reproductive and vegetative growth occur simultaneously and, consequently, both developmental stages compete for resources provided by either reserve mobilization or photosynthesis (Blumenfield et al, 1983; Scholefield et al., 1985; Wardlaw, 1990). In avocado, early fruit abscission has been attributed to low carbohydrate reserves in the trees (Davie et al., 1995) and fruit drop has been shown to be accompanied with the reduction of carbon export from the leaves (Finazzo and Davenport, 1986; Finazzo et al., 1994). The presence of starch in the ovary has been reported at anthesis (Sedgley, 1979), but the implications of starch reserves in the reproductive process have not been analyzed. In an attempt to elucidate the causes of high flower abortion in avocado we have analyzed the role of nutritive reserves of the flower on fruit set with an image analyzing system attached to the microscope (Rodrigo et al., 1997) that allows detecting and measuring differences among flowers (Rodrigo et al., 1997). The results show that, whereas no external differences are apparent among flowers at anthesis, a wide variability exists in starch content at flower opening that could be related to the abscission rate since a very small proportion of the flowers present high starch

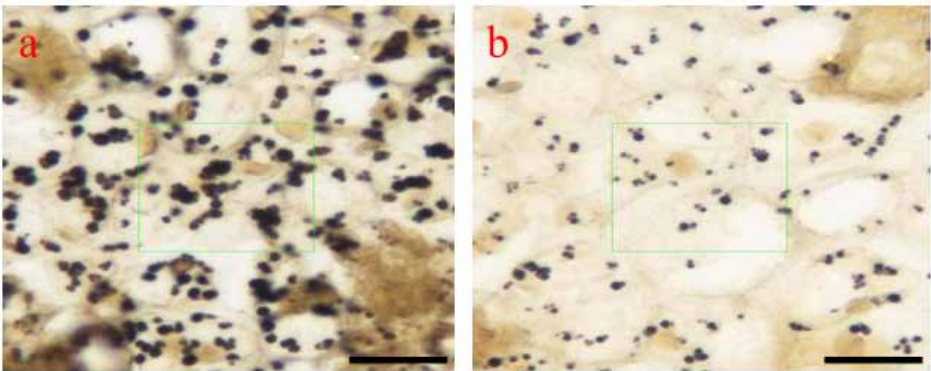


Figure 4. Avocado ovary transversal sections stained with I_2KI in two flowers with high (a) and low (b) starch content. Bars 20 μ m.

content in the ovary (Fig. 4). In order to establish whether the nutritional status of the flower at anthesis is related to the reproductive success of each flower, starch content in the ovary was measured in two populations of flowers with different capacity to set fruit: flowers opening during April and others opening at the end of May not resulting in fruit set. Significantly higher starch content was recorded in the flower population more likely to set fruit supporting the idea that flowers with the highest starch content at anthesis could present some advantage to develop into fruits.

As a next step, based on the existence of the relationship at anthesis between starch content in the cortical tissue of the style and starch content in the ovary, an experiment was performed in order to establish the implications of nutritive reserves accumulated in the flowers on reproductive success. With this purpose, 40 'Hass' flowers were hand-pollinated daily using pollen from 'Fuerte'. After pollination, each flower was labelled and the style was cut off the following day once the pollen tubes had grown along the style and fruit set was examined in the field. This method allowed relating the fate of ovules that remained in the trees with the nutritive reserves in the flowers at anthesis. The results obtained in this work demonstrated a correlation between starch content in the flowers at anthesis and fruit set suggesting that the capacity of a flower to become a fruit could be preconditioned by the nutritive status of the flower. High starch content at anthesis would thus be a necessary although not an exclusive condition for fruit set.

The combination of all these experiments indicates that multiple factors are responsible for the low fruit set in avocado and that a holistic approach should be followed in order to optimize avocado fruit set. On the one hand, the opportunities for pollination should be increased by optimizing the density of honeybees in the orchard. Moreover, taking into account that the floral behaviour is very sensitive to environmental conditions (temperature and relative humidity) it could be interesting to design mixed plantation systems involving two or more pollinizer genotypes that would have the advantage of decreasing the possible negative effect of a lack of synchrony depending on the environmental condi-

tions. This approach planting different pollinizer genotypes in the same place is already being used with success in some orchards (R. Hofshi, personal communication). Ideally those pollinizers should produce Hass-like fruits to increase the market value of the final product. Moreover, although avocado has been described as a species with a marked protogynous dichogamy, it is common to observe flowers in different sexual stages at the same time among trees of the same genotype and even within the same tree allowing self-pollination. The influence of the starch reserves of the flower at anthesis on fruit set suggests that the nutritive status of the tree and the allocation of resources to the developing inflorescences and flowers could play an important role on fruit set, being a necessary although not sufficient condition for fruit setting.

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