Enhancement of Avocado Pollination and Fruit Yield

Continuing Project; Year 2 of 2

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Benefits to the Industry

Pollination is a factor limiting yield in commercially grown avocados (Ish Am and Eisikowitch, 1991, 1993; Vithanage, 1986). Italian honey bees are typically brought into orchards to carry out pollination. However, their low attraction to avocado bloom means that alternative forage, such as citrus and wildflowers (Stout, 1923; Ish Am and Eisikowitch, 1992), competes strongly for honey bees' pollination services. In an attempt to identify a pollinator that is more strongly attracted to avocado than the commonly used Italian honey bee, we have been evaluating honey bees of the New World Carniolan (NWC) race. Some of our data from last year, as well as that of our collaborators in Israel, Sharoni Shafir and Arnon Dag, suggest that NWC honey bees may be more effective pollinators of avocado than Italians, at least in some settings (Fetscher et. al, 2000).

Also of great concern to the California avocado industry is cross pollination. The synchronously dichogamous flowering behavior characteristic of avocado is thought to discourage selfing. For this reason, growers often include pollinizer cultivars in their orchards, in order to promote outcrossing between trees of different flower types. However, the most popular pollinizer cultivars used at this time produce fruits that are generally considered inferior to 'Hass'. "B"-flower-type cultivars that both served as effective pollen donors and produced more marketable fruit than existing pollinizers would be of great benefit to the California avocado industry.

Objectives

This project seeks to enhance the avocado industry through the improvement of avocado pollination. We have focused our efforts on two factors which potentially influence pollination and fruit set: 1.) pollinator type, and 2.) pollen donors.

1. The goal of the pollinator component of this project is to compare the visitation rates of nectar foragers of the Italian- and New World Carniolan (NWC)- races of honey bee to avocado bloom, relative to competing forage, and ultimately to determine whether NWC is a superior pollinator of avocado.

2. The goal of the pollen-donor component of the project is to compare 'Hass' and several pollinizer cultivars, including some new ones (from the UC Breeding Program) that produce 'Hass'-like fruits, with regard to their ability to donate pollen to 'Hass' flowers and contribute to 'Hass' yield. Such information is an important component in decision-making about which pollinizer(s), if any, merit interplanting in 'Hass' groves. In addition, we are compiling information about the floral biology of the various cultivars that could underlie the observed variation in 'Hass'-fruit siring ability among them. We have undertaken a very comprehensive study of the flowering phenology, both at the scale of the season, and day-to-day, across cultivars to determine how well pollen shedding of each overlaps with the pollen-receptive stage in 'Hass' flowers in different settings and under different climatic conditions in California. Ultimately, it will be of use to compare the overlap of the male phase of the various pollinizers and 'Hass' female phase to the level of male/female overlap that occurs within

'Hass' itself, thus establishing the degree to which self pollination can be depended upon, and how much the availability of outcrossing pollen might increase the level of pollination possible.

Summary

1) Alternative pollinators for avocado (NWC honey bees)

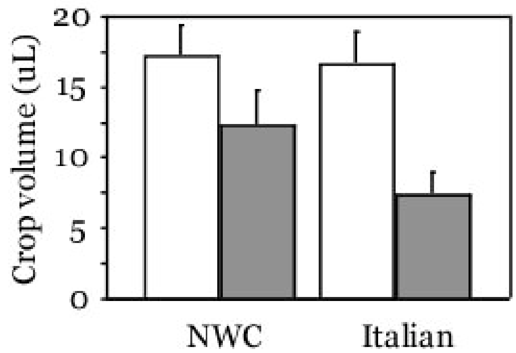
In order to estimate the frequencies of visitation to avocado by Italian and NWC bees when competing forage was present, we set up apiaries in two locations: the ACW Farm in Fallbrook and the Debusschere Farm in Oxnard. Both locations have alternative forage nearby, although it differs markedly in type, quantity, and layout. In Fallbrook, the study apiary was set up on the edge of the grove where wildflowers such as lilac, mustard, sumac, buckwheat, wild radish, deerweed, and lupine could be accessed easily by the bees. In Oxnard, rather than wildflowers, blooming eucalyptus, lemons, and strawberries were in close proximity to the avocado planting. Study plots in each site contained equal numbers of NWC and Italian hives (for a total of 40 in Fallbrook and 60 in Oxnard). All colonies of both races had been established by naturally mated queens purchased from Heitkam's Honey Bees in September of 2000. NWC pools from this supplier are "recharged" regularly with genetic material from the NWC Breeding Program at Ohio State University (Cobey and Lawrence 1988, Cobey 1999). In preparation for the experiment, colonies each race were roughly equalized by transferring 4 full frames of brood (and bees) per colony into new hive boxes (along with 3 frames of honey and 3 empty frames), and adding a second, empty hive box ("super") on top, for the accrual of new honey. Colonies were moved to the Oxnard site on 29 March 2001. The remaining colonies stayed in Fallbrook.

a) Honey bee visitation rates to avocado – individual bees

In order to infer visitation to avocado flowers by individual nectar foragers at both study sites, bees were caught on their way back to their hives and the contents of their crops (honey stomachs) were collected for eventual laboratory analysis for the presence of perseitol (a 7-carbon sugar unique to avocado, which can be found in avocado nectar). On each day of this part of the study, 10 bees/hive from an equal number of randomly selected hives of each race were sampled at varying times throughout the day between 8 am and 6 pm. We captured returning foragers at the hive entrance with an insect net and chilled and anaesthetized them briefly with dry ice. Their abdomens were then gently squeezed to purge the contents of their crops. The entire volumes of the nectar they had collected were taken up and measured using Drummond® glass microcapillary tubes. These samples were then diluted in water, quick frozen in the field on dry ice, and are currently being analyzed by HPLC to ascertain whether perseitol is present and determine whether there are differences between the honey bee races in visitation rates to avocado. Rick Miranda of Dr. Monica Madore's lab at UCR is carrying out the laboratory work.

Some recently analyzed data from last season have revealed striking differences between the honey bee races in the volumes of nectar of their crops upon return to the hive after foraging at avocado. At the Orr farm in Somis (where we had a study apiary in the 2000 flowering season), foragers of both races that had visited avocado had lower crop volumes than those that visited other nectar sources (Fig. 1). The crop volume of NWC foragers returning from avocado was reduced by 30% ($F = 1.65$ (nectar source), $F = 1.41$ (hive); $P > 0.2$ for both nectar source and hive; two-factor ANOVA), relative to those visiting non-avocado flowers. Interestingly, Italian foragers exhibited a significantly greater reduction (58%) in crop volume when returning from avocado relative to other nectar sources ($F = 4.35$, $P < 0.043$ (nectar source); $F = 2.04$, $P < 0.039$ (hive)). Among avocado foragers, NWC foragers tended to return with greater crop volumes than Italians, although the difference was not significant ($F = 1.80$, $P > 0.18$). These data show differences in the two races with regard to their visitation behavior to avocado. Our results could mean that NWC bees are either imbibing more nectar per avocado flower than Italians, or, more plausibly, they are visiting more flowers per trip, relative to Italians, when foraging at avocado. Either possibility could be the result of a higher preference or tolerance for avocado nectar among NWC bees compared to Italians. This finding is of interest because, if NWC bees indeed visit more avocado flowers per foraging trip than Italians, there could be a greater chance that the former bees will move more between trees and thereby possibly effect more cross pollination than Italians, in groves containing both "A" and "B" flowering-type cultivars.

Fig. 1. The total volume (+SE) of crop contents of foragers from Italian and New World Carniolan colonies placed in an avocado orchard at Somis, CA, caught upon return to their hives. The type of bloom visited by a given forager was inferred by the presence or absence of perseitol in the crop sample.
☐ non-avocado foragers ■ avocado foragers



b) Honey bee visitation rates to avocado – at the colony level

Honey was harvested separately from each of the study hives at both sites at the end of the avocado bloom for eventual estimation of the proportion of honey collected over the season that was from avocado. Avocado honey is very dark and contrasts well to the other honeys typically derived from competing forage. Shafir and Dag have shown that there is a strong correlation between the absorbance of light at 260 nm and the percent of perseitol in honey (unpublished data). Therefore, we will be estimating the relative levels of avocado honey in isolates from all study hives using spectrophotometry. A race-specific difference in absorbance of these honeys will indicate a difference between the races in visitation to avocado flowers, relative to other forage, over the time span of an avocado blooming period.

2) Siring ability of pollinizer cultivars

a) Effect of different pollinizer cultivars on 'Hass' yield

A pollinizer trial was set up in Oxnard, CA by M. L. Arpaia in 1998. The goal of the trial was to test 'Hass'-fruit siring ability of newly generated pollinizers from the UC Riverside Avocado Breeding Program. The pollinizers to be tested had been selected by virtue of their production of dark, 'Hass'-like fruits, which are potentially more marketable than fruits of existing pollinizer cultivars. The trial grove contains 6 blocks of 'Hass' with rows of a different pollinizer interplanted every 6^{th} row of 'Hass'. The following pollinizers are included: 'Bacon', 'Ettinger', 'Zutano', 'Fuerte', 'Marvel', 'Nobel', 'SirPrize' and 'Harvest' (an "A"-flower type). The Breeding Program recently generated the latter four cultivars whereas the former 4 are long-used "standards". The focal 'Hass' trees whose fruit were to be counted were spaced at varying distances (rows) from their nearest pollinizer. On 27 March 2001, a 340-tree subsample of the 'Hass' in the Oxnard trial plot was strip-picked. In conjunction with Dr. Arpaia, we gathered data including the yields per tree and weight of each tree's crop. These data show that the cultivar of the nearest pollinizer and the distance of that pollinizer from the focal 'Hass' tree were both highly significant determinants of 'Hass' fruit count ($P < 0.0001$ for each; Fig. 2). There was also a significant interaction between pollinizer and distance ($P < 0.0092$) on 'Hass' yield. The data demonstrate that a high variability in siring ability exists among the cultivars and that at least one of the new B-flower cultivars with 'Hass'-like fruit ('Marvel') is also an apt pollinizer, as 'Hass' fruit count in its near vicinity was significantly higher than background levels (estimated using values for 'Harvest', an "A"-flower type that served as a control in this study, $P < 0.0190$). Similar results were observed in the harvest weights. There were no significant effects of pollinizer on average individual fruit weights.

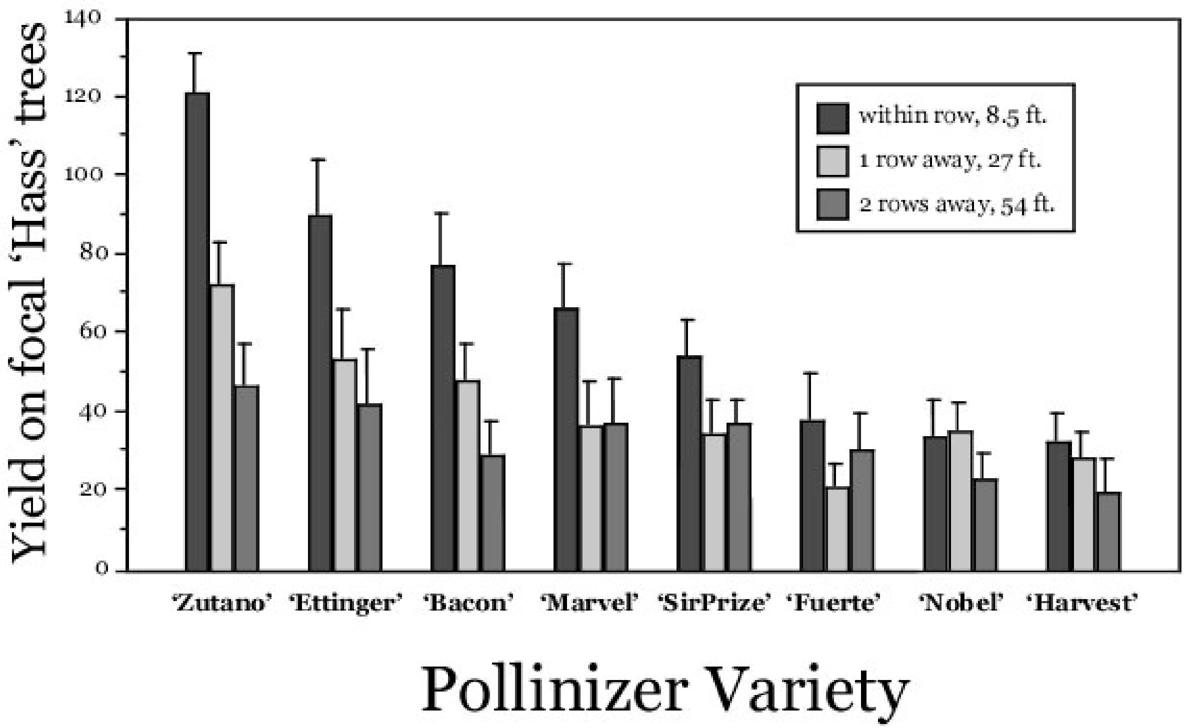


Figure 2. The influence of pollinizers on 'Hass' yield. Fruits were harvested from 'Hass' trees located at varying distances from interplanted rows of pollinizers on the Debusschere ranch in Oxnard, which had contained honey bees at a density of 2.5 hives/acre during the bloom. Bars indicate 1 standard error. It is uncertain whether 'Fuerte' flowered during this season. 'Harvest' is an "A"-flower-type cultivar (i.e. not a pollinizer, *per se*). 'Marvel', 'Nobel', and 'Harvest' are new cultivars from the UC breeding program and currently undergoing evaluation.

b) Flowering behavior of various cultivars – potential consequences for self- and cross-pollination of 'Hass'

As a complement to our work estimating pollinizer effects on 'Hass' yield, we have conducted an investigation of pollinizer attributes that may contribute to their relative abilities to sire Hass fruits. We recorded timing of the onset and end of the flowering season for each of the cultivars in three sites, which are likely to exhibit very different climates. Our study included 'Hass' and all pollinizer cultivars at the Oxnard pollinizer trial, which represents a coastal region, 'Hass' and 'Zutano' at Rancho Simpatica in Fillmore, an inland site, and 'Zutano', 'SirPrize', 'Marvel', 'Nobel', and 'Lamb Hass' at the Weirheim ranch in Moorpark, a geographically intermediate location. In addition, on several days spaced throughout the season at each site, we recorded detailed data on the intraday timing and nature of floral events that are likely to influence the exchange of pollen between male flowers of a given cultivar and female 'Hass' flowers. Data loggers were placed at each site in order to retrieve climatic information during the flowering season, thus allowing the exploration of possible relationships between weather and floral events.

Every 2-3 weeks, starting in early January, we monitored the flowering phenology of a large sample of 'Hass', as well as pollinizers, in our various study sites. At each observation session, trees were scored for flowering stage based on the method used by Salazar-García et al. (1998). There was reasonable variation between cultivars in the time of onset of flowering, yet all cultivars overlapped, at least to some degree, with 'Hass' at all sites during this last season. Figure 3 shows data from the Oxnard site taken on 14 March 2001. By mid April, nearly all trees of all the cultivars were in bloom at this site, and continued at least into May. By the end of May, 'Harvest', 'Bacon', 'Lamb Hass' and 'Zutano' had recently finished flowering, SirPrize and Ettinger were very nearly done, and Fuerte, Marvel, and Nobel were still blooming strongly. Also, at this time, nearly 60% of 'Hass' trees were done flowering. Overall, 'Marvel' appeared to flower over the longest period in Oxnard this past season. Moreover, it overlapped well with the latter stages of the 'Hass' bloom, when, probably due to climatic factors 'Hass' fruitlets were more likely to be retained.

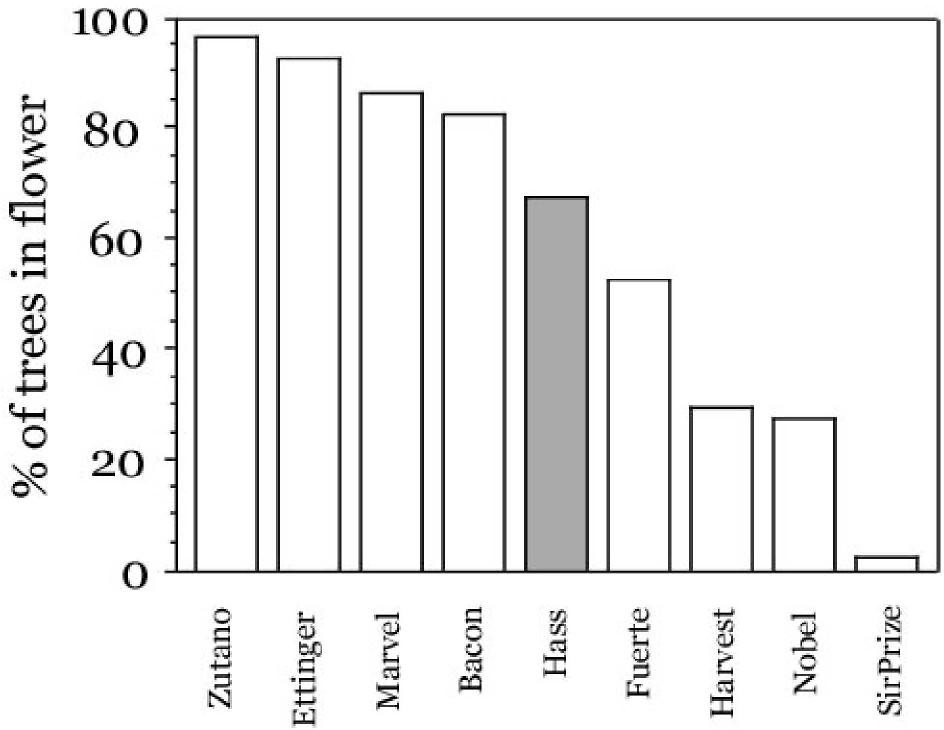


Figure 3 . The percent of trees in bloom on 14 March 2001 at the Oxnard pollinizer trial.

For the individual flower observations, we employed the following protocol: Each day of this part of the study, each observer (A. Fetscher and S. Mills) followed the flowering behavior of one 'Hass' tree and (in Oxnard and Moorpark) one tree of each of 2 or 3 pollinizer cultivars. Starting early in the morning, on each tree, 2 inflorescences at opposite ends of the tree were marked off with tape. Four or more open flowers, if any present, were selected at random and tagged. If flowers of both sexes were present, equal numbers of both sexes were tagged. All remaining untagged flowers were removed and their quantity was recorded. The sex phase, color of the stigma (an indicator of receptivity to pollen), the approximate amount of nectar present (indicating attractivity to pollinators), the number of anthers dehisced, and the presence of pollen, were all recorded for each tagged flower. Each inflorescence was revisited approximately every 1 to 2 hours throughout the day. During each visit, existing tagged flowers were observed and the abovementioned data were recorded again, and four or more new flowers (if present) were tagged and their data were also recorded. Again, any additional flowers were removed and their quantity was recorded. Closure of tagged flowers was also recorded as it occurred. This protocol allowed us to gather data not only on timing of female and male floral openings and closings but also the duration of the sex phases of each flower and the extent of overlap of male and female phases both between 'Hass' and the various pollinizers and within individual 'Hass' trees. The data we collected are also important in determining the possibility of 'Hass' self pollination in male-phase ("stage 2") flowers, which is a central focus of our collaborator, Tom Davenport's, portion of this pollination study.

The result of our flower-observation work is a massive data set consisting of over 5,000 floral observations from March through late May. To date, only the 'Hass' data have been completely processed. A summary of the findings in this cultivar, showing periods of overlap between pollen-producing male flowers and receptive female flowers is provided in Figure 4. Temperature and humidity data corresponding to the flower-observation-study dates are shown in Figure 5. The frequency of occurrence of white (receptive) stigmas in 'Hass' flowers at the Oxnard and Fillmore sites on the study dates are shown in Figure 6. The 'Hass' flowering summary reveals wide variation in floral behavior from day to day during the season. More analyses must be conducted before any conclusions can be reached about specific temperatures' and humidities' effects on flowering in our climate, however, several points are readily apparent when viewing these data. The female phase in 'Hass' can be skipped completely, given the right conditions (as evidenced by the 4/11 data from Oxnard). Male flowers can, and often do, stay open all night long, and can remain open briefly, the following morning, and continue donating pollen. There are sometimes even two distinct male phases within a given day (as seen on 5/15 in Oxnard, and, interestingly, two of the three observation days in Fillmore). Periods of overlap between male and female phases within 'Hass' range from $\leq$ 0.5h (as in the early-season observation days in Oxnard) to nearly the whole day long (as in the early-observation day in Fillmore). It should be noted, however, that all of the periods of male/female overlap are to be considered the *maximum* possible for each day, because they include the extreme limits of the periods during which flowers of each sex phase co-occur. Since opening and closing of each sex phase generally occurs over a long period (as shown by the ranges indicated by parentheses on the schematic), only a (usually small) subset of flowers of a given sex phase is open during these endpoints. Therefore, the indicated overlapping periods should be treated as upper limits of the extent to which overlapping of sex phases, and therefore the capacity for pollen transfer between stage-1 and stage-2 'Hass' flowers, can occur.

The occurrence of white (receptive) stigmas on male 'Hass' flowers is thought to be a factor determining the degree to which selfing can occur in the male phase. Understanding the basis for color difference in stigmas is an important factor in determining the possibility for self pollination in the male phase and therefore the need for pollinizers and perhaps even pollinators in a given setting. The implications of stigma color are dealt with in more detail in Dr. Davenport's portion of the pollination project.

There are at least two hypotheses explaining the underlying cause of stigma senescence (i.e. when a white stigma turns brown and is therefore no longer receptive to pollen). The stigma may turn brown faster if ambient conditions are dry enough (low humidity), or the stigma may turn brown as a result of pollen receipt. The latter possibility has been demonstrated in other plant species (Procter *et al.*, 1996). The former hypothesis is not well supported by our data. On 5/4 in Oxnard, when there was a dramatic dip in humidity relative to the other study days at this site, the proportion of white stigmas in late-stage male flowers fell to zero, which corroborates the humidity hypothesis. However, data from Fillmore showed the opposite trend. That is, on the less humid day there was actually a higher proportion of white stigmas in the male stage. It should be noted, however, that our sample sizes at this point are too small for statistical analyses of any relationship between stigma color and humidity, and therefore our ability to rule out the importance of humidity is limited. With regard to the pollination hypothesis, inside Dr. Davenport's cages, we have conducted some hand pollinations of about-to-close female flowers bearing previously unpollinated, white stigmas and found that, in all cases, the stigmas were already starting to turn brown when the flowers were reopening at the onset of the male stage. In contrast to this, all the control, unpollinated stigmas in the cage were still white upon the second opening. These results provide some support for the importance of prior pollen receipt for early senescence of the stigma. However, it is not yet clear if pollination can completely explain stigma senescence. We collected pistils (female organs) from a large number of 'Hass' flowers in the field and recorded stigma color upon collection. We have begun viewing some of the pistils under the microscope in an effort to count pollen grains and pollen tubes and see how far down the pistils the tubes have advanced as a estimator of the time elapsed since pollen receipt. If the pollination hypothesis were correct, there should be an increasing tendency for brown-stigma pistils to have pollen and fairly advanced pollen-tube growth relative to white-stigma pistils. From the few samples that have been examined so far, no clear relationship between these factors is evident. However, many more specimens have yet to be examined.

Figure 4. Schematic of 'Hass' flowering behavior on selected days at the Oxnard and Fillmore study sites. Black lines represent female ("stage 1") flowers and grey lines are male ("stage 2"). Circles indicate timepoints at which flowers were opening. X's indicate timepoints at which flowers were closing. Parentheses indicate when there is a range in the period of time during which flowers of a given sex on a given tree are opening or closing. For practical purposes, only functionally male flowers (i.e. ones that have begun releasing pollen) are included in the schematic. Shaded boxes indicate periods of overlap between the sex phases, such that male flowers on a given tree could potentially donate self pollen to female flowers on that same tree (or, presumably, others of like cultivar in the orchard).

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