Are Thrips Important Pollinators of Avocados in California?

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The importance of honey bees as pollinators of avocado flowers is a contentious issue. The reason for this is that there is no clear or consistent experimental evidence unequivocally demonstrating that honey bees are essential for pollinating avocado flowers and promoting maximum fruit set. While many different types of insects are known to visit avocado flowers (e.g., beetles and flies) and it is assumed that these insects play some role in pollination, their exact importance is not known. One group of insects, thrips, in particular flower thrips (i.e., *Frankliniella*), are commonly found in avocado flowers during bloom. These tiny hairy insects have not been specifically studied for their ability to act as pollinators of avocado flowers. It is very likely that given the small size of these insects, their hair covered bodies, their high numbers and almost constant presence in flowers, and predilection for feeding on pollen and nectar, they may be important, but overlooked pollinators of avocado flowers. If this is the case, then conflicting conclusions resulting from bee studies using exclosure cages may be explained by thrips moving pollen within and between trees. Thrips pollination could occur because cage mesh used to exclude honeybees is not small enough to prevent thrips and other small insects access to flowers, thus allowing pollination to occur either by thrips resident on trees at time of caging, or moving between trees. Consequently, equal rates of fruit set between caged and uncaged trees could be explained via the thrips-pollinator hypothesis, rather than the assumption that wind pollination was the primary cause for self and cross-pollination. Therefore, the objective of this proposal is to ascertain the role that ubiquitous flower thrips, especially *Frankliniella* spp. play in pollinating 'Hass' avocados in California and its significance relative to the role of wind and honeybees.

This project originally requested $46,478 to conduct this work. The PRC awarded the proposal $5,000, or 11% of what was needed to run the proposed experiments. To make up the significant shortfall, The Hofshi Foundation, stepped up and generously helped to support this "preliminary investigatory" research by: (1) purchasing three large mesh exclosure/enclosure cages with zippered-door antechambers large enough to cover 14 four year old avocado trees (each enclosure had ~12 Hass trees and ~1-2 pollinizer trees), seven trees in each of two rows, at ACW Ranch in De Luz, CA (Figure 1A and 1C). (2) Design and construction of a PVC framework to support the exclosure/enclosure tents insuring that the enclosures did not touch the experimental trees (Figure 1B). (3). Design of a unique PVC pole system to hold six glass microscope slides coated with Vaseline to capture airborne avocado pollen moving between and within study plots (Figure 1D). (4) Provision of a labor crew to install the PVC framing and mesh cages. (5) Hiring of a field assistant to monitor and score avocado bloom development. (6) Installation of four state-of-the-art Davis Instruments Wireless Vantage Pro 2 Plus weather stations; one in each of the three tents and one in the control plot to measure temperature, humidity, wind speed, UV and solar radiation. (7) Two Tauber traps per treatment to capture airborne pollen that drops to the ground (Figure 1E). (8) Hiring of a post-doctoral researcher at UCR to stain aerially-dispersed-pollen-collection slides and count avocado and other pollen on stained slides under a microscope to determine the role of wind in moving pollen between avocado trees in each of the four experimental plots.

Figure 1. (A) Above shows two of the three large mesh exclosure/enclosure tents each covering 14 avocado trees at ACW Ranch in De Luz, CA. **(B)** Inside the tents showing the experimental plants (seven trees in two rows) and framework to support the mesh. **(C)** Showing antechamber with zippered entrance to protect enclosed trees from unwanted insect ingress. **(D)** Poles with microscope slides (6 horizontal positions) and 1 vertical position) to catch airborne pollen moving between trees from either inside or outside the enclosure. **(E)** Custom made Tauber trap to catch pollen dropping from trees.

To investigate the role of thrips in the pollination of avocados in California, five treatment plots each consisting of 14 trees (two rows of seven trees) were established at ACW Ranch in February 2010, well in advance of bloom that commenced in mid March 2010. At the initiation of the project all weeds in the study areas around trees selected for this study were hand removed. All fruit were also harvested from the trees prior to treatment establishment. The five treatments were: (1) Control Treatment – no water spray; this consisted of uncaged plants and fruit set was left to occur under prevailing orchard conditions (i.e., visitation of small insects and honeybees, and the effects of wind etc were not influenced in anyway). (2) Control – water spray treatment was set up to determine the effects of water spray applications (without insecticides) on fruit set rates. (3) Insecticide Treatment; trees enclosed in a Polysac Crystal 17% (light scattering) mesh tent (mesh size,1.44 mm^2) were sprayed on an as needed basis with malathion or danitol to kill all small insects (e.g., thrips) on trees to prevent pollination by small insects. The small mesh size prevented larger insects (e.g., bees) accessing flowers. Fruit set in this treatment, should it occur, could suggest that wind pollination, gravity or tree movement was responsible. (4) Thrips Treatment; trees were enclosed in a Polysac Crystal 17% (light scattering) mesh tent (mesh size, 1.44 mm^2) to exclude all large insects like flies, beetles, and bees that could visit avocado flowers and assist with pollination. Fruit set in this treatment could be the result of pollination by small insects moving in and out of the cage and wind (the contribution of wind to fruit set will be determined from the insecticide treatment cage, and presumably would be additive in effect to small insect activity in this treatment). (5) Bee Treatment; this enclosure cage used Polysac Crystal 10% shade (light scattering) net which had 5 mm^2 mesh openings that excluded large insects like flies and beetles and honeybees. Attached to this cage was a double-entrance beehive and honeybees were able to enter and exit the cage via the hive to forage on flowering avocados inside and outside of the cage on their own accord (they were able to forage on other plants outside the cage, inside all weeds that could have flowered were removed and weeds were not therefore a competing food source).

From these treatments we expect that fruit set in the Bee Treatment would result from bee, small insect activity, and wind. Fruit set inside the Insecticide Treatment cage which excluded all insects will be used to determine the contribution of wind, gravity or mechanical rubbing of branches. Fruit set in the thrips cage will determine contributions to fruit from small insects and wind together. Fruit set in all three cage treatments will be compared to the two control plots, where fruit set occurred in the absence of any experimental manipulations designed to affect insect foraging on bloom. It is anticipated that these five treatments may provide the preliminary necessary data to determine the role of wind, small insects like thrips, and honeybees in fruit set in avocados. At time of harvest in 2011, fruit yields will be compared across treatments to determine effects of wind, small insects, and bees on fruit set rates in Hass avocados in Southern California. The results of this work will have important ramifications for pre-bloom and bloom sprays for control of pest insects like avocado thrips. In addition to pollen capture slides which were replaced weekly at ACW, additional data were collected weekly including rating of flower bloom and insect counts in avocado bloom in all four treatment plots. These data were used to determine if bloom progressed differently across the five treatments and if significant differences in the insect fauna associated with avocado existed between the five treatments.

The weather station data from inside the cages indicated that there was little effect on wind movement, humidity, and temperature inside cages in comparison to the non-caged control plots (data not presented). Light intensity was slightly lower in cages (~1%), but not sufficiently lower so as to affect fruit set as observed in the honeybee cage treatment.

Insect Counts: On a weekly basis, different types of insects were monitored and counted in each treatment. The following groups of insects were monitored: honeybees, big flies, small flies, wasps, thrips, small beetles, and big beetles. Flowers on trees were inspected for large insects by carefully approaching a quadrant and examining it for ~2 minutes recording the number of insects in that particular cardinal quadrant of the tree. Small insects were then counted in the same quadrant on bloom using a hand magnifier (~ 5 mins). Once big and small insects were counted, the next quadrant was examined. This process was repeated until all quadrants had been inspected on a particular tree. A random number generator was used to select seven trees in each experimental block for inspection.

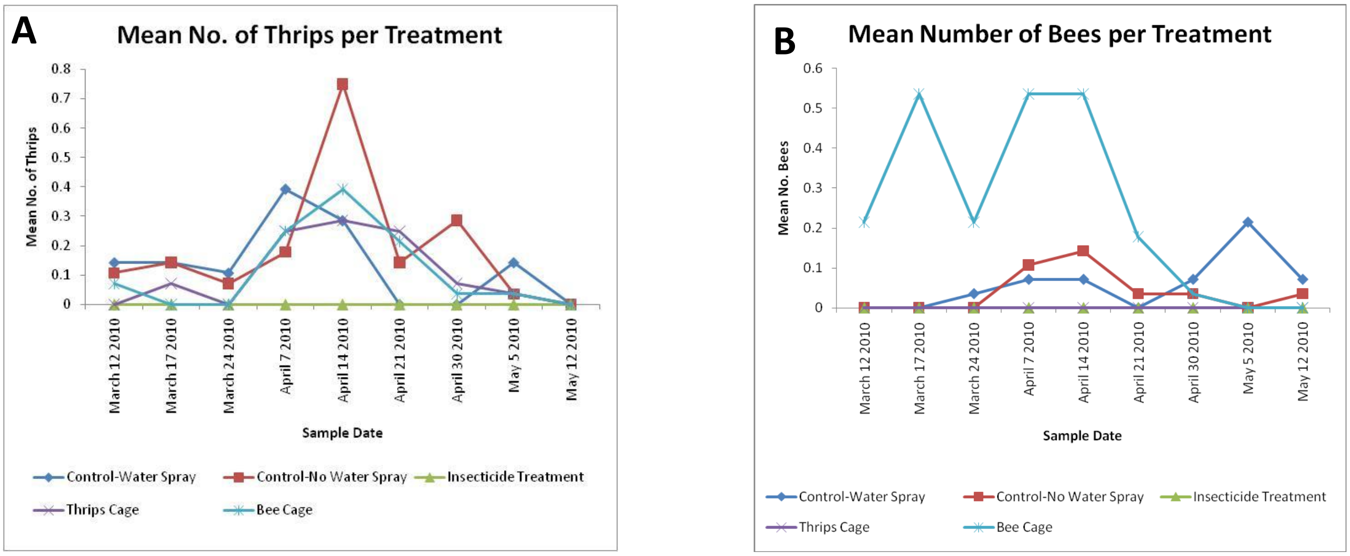


Figure 2. Mean number of Thrips (A) and Honeybees (B) in the 5 experimental treatments.

Figure 2 shows the mean number of thrips (A) and honeybees (B) counted on avocado bloom across the five different experimental treatments. Thrips were fairly consistent in their numbers across all treatments (Fig. 2A). A noticeable peak in the control-no-water-spray was observed on April 14 2010, the reason for this peak is unknown. The insecticide application treatment was very effective at eliminating thrips from avocado flower bloom. Bee activity (Fig. 2B) was similar across all treatments, except in the bee cage treatment, where honeybee activity on bloom was significantly greater.

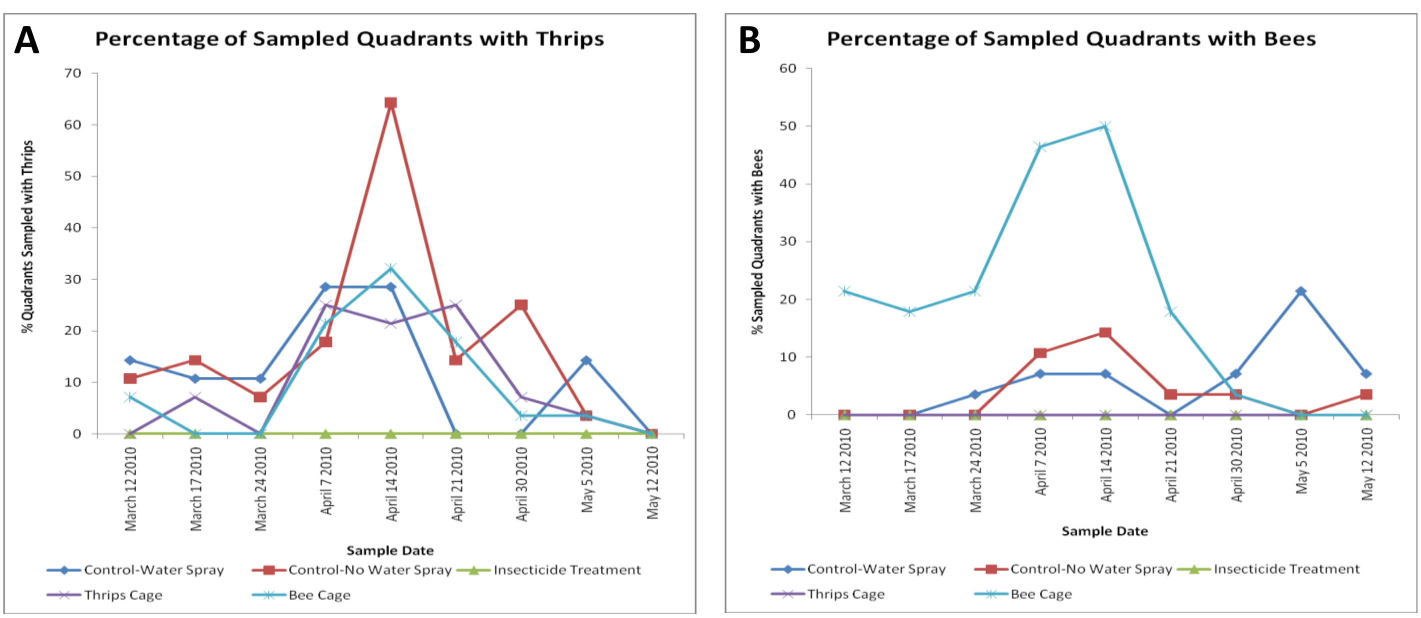


Figure 3. The percentage of tree quadrants with either thrips (A) or honeybees (B) in the 5 experimental treatments.

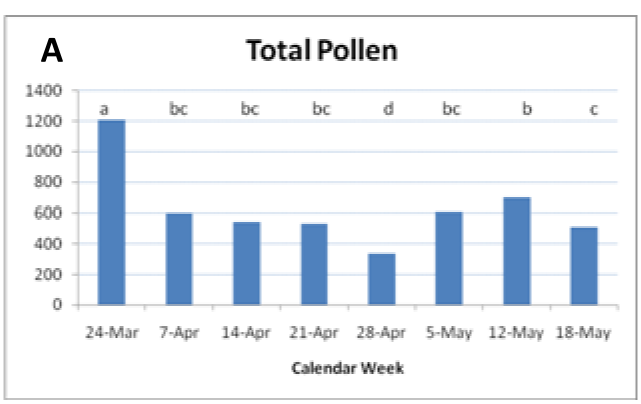
In Figure 3, the percentage of quadrants (i.e., north, east, west, and south sections of trees) with thrips foraging in the bloom was similar across all treatments expect for April 14 2010 when a noticeable peak was seen (Fig. 3A); this timing corresponds with the same peak seen in Fig. 2A. Fig. 3B shows that bee activity in quadrants was also higher in the bee cage (as seen in Fig. 2B).

Similar count data and percentage of quadrants with insects for big and small flies, big and small beetles and wasps, is still being analyzed.

Flowering: We rated all trees weekly for flowering stage. Prior to bloom we rated the trees using the flowering scale developed by Salazar-Garcia et al. (1998). All Hass trees regardless of treatment developed at the same pace and the trees were considered to be commencing bloom the week of April 5 2010. From that time forward we weekly rated trees for the approximate percentage of bloom that had occurred as well as flower intensity. There were no differences between the treatments. Flowering occurred from early April through mid-May. The pollinizer trees (Bacon, Ettinger and Zutano) bloom commenced slightly earlier. Flowering for the pollinizer trees was from late March through mid-May.

Pollen Counts: Once bloom commenced, glass microscope slides were deployed in each treatment (6 poles per treatment and 6 heights per pole (Figure 1D) with horizontally-oriented Vaseline coated slides. We also had 1 vertically-oriented slide per pole. Slides were collected weekly for analysis of pollen deposition. We did not count the pollen from Treatment 2, the control water spray treatment.

In all treatments, very little avocado pollen was collected on the glass slides over the course of monitoring (late March through mid-May). The dominant pollen type found on the slides was pine pollen. Pine trees are wind pollinated and there is one large pine tree and 3-4 small pine trees a few miles from the experimental site. Figure 4 shows the average pollen counts over the course of the avocado flowering period. Fig. 4A shows that average total pollen counts ranged from 1210 pollen grains/week (March 24) to a low of 326 pollen grains/week (April 28). Avocado pollen numbers ranged from 34 pollen grains/week (April 21; during peak bloom) to a low of 3 pollen grains/week (May 18; the end of bloom). The highest average avocado pollen counts were observed in the honeybee cage treatment (24 pollen grains/week). This was significantly greater than the average pollen counts for either the insecticide (12 pollen grains/week) or thrips cage treatment (10 pollen grains/week). The control – no water spray treatment (18 pollen grains/week) was not significantly different from either the honeybee cage treatment nor the pesticide treatment. This finding strongly suggests that wind dispersion of avocado pollen is not a major factor in avocado pollination.



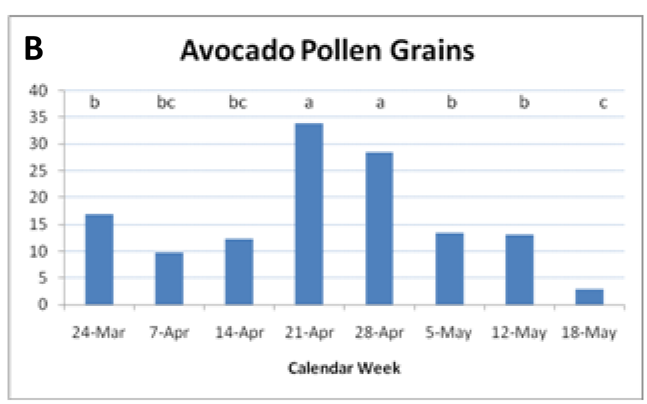


Figure 4. Average pollen counts per slide per week across all experimental treatments. **A.** Total pollen counts on glass microscope slides. **B.** Average avocado pollen counts on glass microscope slides.

Fruit Set: Final data on fruit yield results for treatments is still pending and will be gathered in 2011 when fruit from each experimental tree will be counted and graded. These data will then be compared across treatments to determine treatments effects on yield.

Tentative observations suggest that the following fruit set patterns may be occurring across treatments: (1) Control and water-spray control have relatively high levels of fruit set. (2) The insecticide application treatment appears to have no fruit suggesting wind and self-within the flower pollination is not important for fruit set. (3) The thrips treatment has a very low level of fruit set which may mean small insects make little contribution to fruit set. (4) The honeybee treatment appears to have similar levels of fruit set as seen in the two control plots. This is suggestive that mainly honeybees and some small insects are correlated with high fruit set. However, if the yield in the honeybee house and the control plots are similar, then the higher numbers of honeybees seen foraging on bloom in the honeybee cage (see Figs 2B and 3B) is likely not significant with respect to yield. If this suggestion is correct, then it would appear that avocado bloom can support a lot of honeybee foraging activity but this may not result in significantly more fruit and just as importantly increased revenue for growers stocking orchards with honeybees. This is counter to results from previous cage studies which had honeybees as part of the cage experiment. Our experimental design differed from previous studies in that we had a two-entrance beehive, thus giving the honeybees a choice of either foraging within the cage or outside instead of forcing them to remain on the caged avocado tree. The fact that the honeybees voluntarily went into the cage and visited the avocado flowers is very interesting since avocado growers and researchers argue that honeybees do not like to visit the avocado flower and prefer other more attractive options. Inside the honeybee cage, the only nectar or pollen source were the avocado flowers. If competitive bloom outside of the cage (e.g., weeds) was preferred it did not influence honeybee activity even though outside the cage there were plenty of food sources to forage on.

Additional Activities: Two time-lapse cameras were installed in the bee enclosure, one was focused at the entrance to the beehive and the other was directed at an inflorescence inside the honeybee cage. The purpose of these cameras was to capture bee activity during the day, flower phenology and fruitset, and to correlate these activities with temperature, humidity, wind speed and direction, solar energy, etc. The beehive was photographed every 30 seconds and the inflorescence was photographed at every 30 seconds and later at 5 and 10 minutes intervals.

One very important outcome of implementing this camera technology has been the opportunity to observe and document in addition to pollination, fruit growth, leaf, and shoot growth as influenced by the environment and the crop load. High quality time-lapse photography technology provides continuous observations of physiological processes and compares these visual records to previously published and ongoing research results. For example, compare the 2 time periods shown in Figure 5 on May 11 2010 when the temperature and humidity were similar in both locations. At 11:49 a.m. the solar radiation is at 434 watts/m^2 while at 1:16 p.m. the solar radiation is 1018 watts/m^2. Notice the great difference in honeybee activity at the hive entrance.

Figure 5. A. Honeybee activity and floral stage of Hass at 11:49 a.m., May 11, 2010. **B.** Honeybee activity and floral stage of Hass at 1:16 p.m., May 11, 2010 when solar radiation was more intense.

Lessons Learned from this Preliminary Trial: This work has been extremely instructive because it demonstrated the feasibility of the proposed project on ascertaining the contributions of various agents to avocado pollination and subsequent observed levels of fruit set. As the preliminary trial progressed refinements were made to the experimental design and data collection techniques were optimized. Additionally, opportunities were actively sought to seek criticism and input from pollination ecologists and some who investigate pollination in avocados, this feedback helped immensely in improving the projects goals and the procedures needed to meet those goals. We received very useful input regarding our experimental design and pollen collection methodologies from G. Ish-Am, D. Eisikowitch, A. Dafni, Emi Lahav, Shmuel Gazit and U. Lavi in Israel and Iñaqi Hormaza in Spain.

Take Home Message: We want to understand the role insects play in avocado pollination because management of pollinators has the potential to affect the grower's bottom line. The key question of interest is: *<u>"Is bee hive stocking necessary in avocado orchards?"</u>* If the answer is “**yes**”, it affects the grower’s bottom line because the failure to stock orchards with bee hives results in lower fruit set and less income. If the answer is “**no**” honeybees are not needed but hives are put in orchards anyway, the grower has wasted money on a service that was not necessary. Further, if small insects like thrips are important pollinators of avocado flowers, then it would be inadvisable to kill flower thrips with insecticides if they are providing free and effective pollination. This issue could be particularly problematic if insecticides are applied at bloom for the control of avocado thrips.

Ongoing Work and Future Plans: We are currently repeating this trial in Chile but with 5 replications of each treatment. The Chilean project is being conducted on a large high density Hass orchard near Panquehue and is being funded by the Hofshi Foundation, Jorge Schmidt (the grower) and GAMA (a private consulting firm). We would like to also repeat this large-scale study, depending on funding, in California in Spring 2011. This would enable us to build upon the progress made in the preliminary trial reported on here that was conducted at Farm ACW, and to compare California results from a comparable trial to those from Chile where this experiment that is currently underway.