

The role of insect pollinators in avocado (*Persea americana*) pollination in New Zealand and Australia

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Abstract

While fruit set on avocado trees is generally less than 0.3%, research in New Zealand and Spain has shown that hand pollination can produce fruit set around 5%. Understanding why the application of pollen grains to avocado flowers markedly increases fruit set will enable us to improve the efficacy of pollination systems. Enhanced pollination in OFF flowering years (when there are reduced numbers of inflorescences) has the potential to help to mitigate the effects of alternate bearing. The current study is being conducted in both New Zealand and Australian orchards to determine the types and numbers of insects visiting avocado flowers, the time after anthesis at which the flowers were visited, the numbers of pollen grains transferred, and the proportion of insect visitors carrying avocado pollen. In New Zealand, 97.4% of flower visits were by honey bees, while in Australia 49.7% of the visitors were hover flies, 37.9% were honey bees, and 12.4% of the visits were from other insects (flies other than hover flies and native bees). In New Zealand, 17.7% of flowers did not receive any insect visits while open during the female phase, and no pollen grain deposition was recorded on a further 68% of visited flowers. In Australia, 100% of flowers observed at the female stage received an insect visit and pollen was transferred. However, the total number of pollen grains transferred onto female phase flowers by visiting insects was low in both countries, c. 4 and 5.9 pollen grains in New Zealand and Australia respectively. The low number of insect visits (New Zealand) and pollen grains transferred to flowers may be factors that limit pollination and consequently fruit set in these countries.

Introduction

Avocado (*Persea americana*) is an evergreen subtropical fruit tree, native to Central America and Mexico. It is grown commercially in the warmer, northern parts of New Zealand and in many parts of Australia, including South Australia, Victoria, south-western New South Wales, and Western Australia. In New Zealand 'Hass', the predominant fruiting cultivar, is sometimes planted with pollinizers, such as 'Bacon', 'Zutano', and 'Fuerte'. 'Hass' are also grown in blocks without pollinizers. The latter planting design is more common in Australia.

Each mature 'Hass' tree produces approximately eight thousand compound or branched inflorescences, each comprising 100-150 flowers, located in axillary or terminal positions on flowering shoots. A flowering shoot may contain one or several inflorescences. An inflorescence can be determinate (terminating with a flower bud), or indeterminate (terminating with a vegetative bud). Flowers are protogynous (stigma becomes receptive before pollen is released) and dianthesis (each flower opens twice). Flowering within a tree is synchronous (Ish-Am & Eisikowitch, 1993). The synchronous nature of dianthesis in the avocado flowers is sensitive to environmental conditions (Davenport, 1986; Ish-Am & Eisikowitch 1991).

Pollination usually requires an insect vector (Ish-Am and Eisikowitch, 1993). Avocado flowers are visited by a wide range of insects including bees, flies, wasps, beetles, and thrips (Vithanage 1990). In most countries, the European honey bee (*Apis mellifera*) is considered the principal pollinator. In New Zealand, it is recommended that four to 10 hives of honey bees per hectare are introduced for pollination (Avocado Industry Council, 2006). Avocado flowers can be cross pollinated (from flower to flower on different trees), close pollinated (from flower to flower on the same tree) or in certain conditions, self pollinated (within the same flower). Close pollination occurs during the tree's bisexual phase, when female phase

flowers are closing and male phase flowers are opening (Ish-Am & Eisikowitch 1991, 1993). Shoval (1987) showed that although a stigma only requires one pollen grain to penetrate the ovary for pollination to occur, 20 or more pollen grains are required to ensure a substantial fertilisation rate.

Avocado trees typically produce more than one million flowers, of which less than 0.3% set fruit (Blumenfeld and Gazit, 1974; Blanke & Lovatt, 1993; Dixon & Sher, 2002). Slight variations in the percentage of fruit set often results in trees producing too many fruit in an 'ON' year and very few in an 'OFF' year, a phenomenon termed alternate bearing. This alternate bearing can be a problem for growers and suppliers of avocados because production numbers are not stable. If pollination could be enhanced in 'OFF' years, this biennial swing in productivity could be countered to some degree. In order to assess whether pollination could be increased in 'OFF' years, it is first necessary to better understand how pollination occurs in avocado orchards.

Previous researchers (Vithanage 1990, Ish-Am & Eisikowitch 1995) have estimated pollinator density in avocado orchards and correlated this to pollination and/or final fruit set. They suggest that pollination may be a limiting factor when insect activity is low and/or cross-pollination efficacy or efficiency is poor.

Research in New Zealand (Evans et al., 2010) and Spain (Alcaraz & Hormaza 2009) has shown that hand pollination can produce a higher percentage fruit set; indicating that a lack of pollination may be one of the limiting factors in avocado production. However, hand pollination has not increase fruit set over 6%, which suggests that pollination is not the only reason for the low percentage fruit set.

To determine the reason of the apparent sub-optimal pollination, the current study looked at natural pollination in orchards in Australia and New Zealand. We recorded the types and numbers of insects visiting avocado flowers, the time after anthesis at which the flowers were visited, the numbers of pollen grains transferred by insects, and the proportion of bees carrying avocado pollen (pollen counts for other insects have not been included in this paper). This information will allow us to assess whether there is capacity to increase pollination, and therefore fruit set, of avocado in 'OFF' years, using either native or managed pollinators.

Methods

Studies were conducted in two Australian orchards and one New Zealand orchard. The Australian orchards were located near Hampton, Queensland (Site A), and Port Macquarie, New South Wales (Site B) and the New Zealand orchard was located near Te Puke, in the Western Bay of Plenty (Site C). All blocks were planted with 'Hass'. At Site A, 16 'Sharwill' pollinizers were grown 400m away from the block used, while 'Bacon' and 'Fuerte' pollinizers were planted randomly within rows at Site C. Site B was solely 'Hass'. Site A and C had 4 hives/ha. There were no hives introduced into Site B, but feral honey bee colonies were present in the area. The trees were between two and four metres in height and managed according to industry standards (Avocado Industry Council, 2008). The trials were conducted during flowering in October-November 2010.

Insect visits to flowers

On 26 and 27 October, at Site B, two video cameras were set up to film insect visitation on inflorescences. Newly opening female flowers were marked on each inflorescence with coloured paint, making them individually identifiable. The video cameras were set to record for the duration that the female flowers were open. The duration of video recording was dependent on weather conditions; in the event of rain, filming was stopped. Female phase flowers were marked on two further inflorescences on the same tree. These inflorescences were bagged with fine mesh at the beginning of female's flowering period for control purposes. The approximate times at which both the female and male phase flowers opened and closed was noted. These methods were repeated at Site C between 31 October and 9 November. When analysing the video footage, the numbers and types of insects to visit each marked flower were recorded, as well as the time after anthesis at which the flowers were visited.

We calculated the percentage of flowers to receive a visit and the proportion of visitors that were honey bees or other insects. Flowers that were not filmed for the entire period that the female phase flowers were open were not included in the former.

Pollen deposition

At both Sites B and C, styles of the marked flowers (described above) were excised immediately after the video recording ceased. The styles of the mesh-covered flowers were also excised at this time. Each style was placed on a slide in a cube of gelatine fuchsin. The styles were viewed under a microscope and the number of avocado and other pollen grains counted. For both sites the following was calculated: 1. the percentage of visited flowers that had pollen on their styles; 2. the number of pollen grains transferred/number of insect visits; and 3. for each inflorescence video-recorded, the mean number of pollen grains transferred onto female phase flowers by insects. Flowers that were not filmed for the entire period that the female phase flowers were open were not included in calculations 1 and 3. For Site B we were restricted to using data from one video (a single inflorescence), while data from four videos were used for Site C.

Proportion of insect visitors carrying avocado pollen

On 5 October at Site A, 53 honey bees were caught on female phase flowers between 0800 – 0900 hours. At Site C, 68 honey bees were caught on female phase flowers before 1200 hours, between 27 and 29 October. It is unlikely that any male-phase 'Hass' flowers were open at the time these bees were caught. At each site another 20 bees were caught on male phase flowers. To assess the amount of pollen carried by bees that was available for pollination, the legs of all the sampled bees were removed so as not to include pollen from the bee's corbiculae. The bees were transferred into individual microcentrifuge tubes and mixed with 70% ethanol to remove any adhering pollen grains. The bees were then discarded, the tubes centrifuged, and the excess liquid decanted. Alexander stain was added to each tube to re-suspend the resulting pellet and a known volume of this solution was viewed under a microscope. The numbers of avocado pollen grains present were counted, and the number of avocado pollen grains per bee calculated.

Results

Insect visits to flowers

In Australia (Site B), 49.7% of the visitors were hover flies, 37.9% were honey bees, and 12.4% of the visits were from other insects (flies other than hover flies and native bees), while in New Zealand (Site C), 97.4% of flower visits were by honey bees and 2.6% from brown blowflies.

At Site B, 100% of the observed female phase flowers ($n=7$) received an insect visit, compared with 82.3% ($n = 31$) at Site C.

At Site B, the mean visits/flower was 10, while at Site C it was 2.3. There was a significant difference in the mean visits/flower between days at Site C ($P = 0.005$, unpaired t-test).

Pollen deposition

Pollen was observed on all the visited stigmas at Site B, but only 22% of visited flowers at Site C had pollen. At Site C, a mean (\pm s.d.) of 0.53 ± 1.45 pollen grains were transferred per bee visit. The number of pollen grains per bee visit could not be calculated for Site B, because each flower received visits from multiple species. The mean (\pm s.d.) number of pollen grains transferred per insect visit at Site B was 0.13 ± 0.30 . At Site B, the mean number of pollen grains/visit increased with time after anthesis at which the visit occurred (Figure 1). This corresponds to the time at which male phase flowers were observed to be open (Table 1). At Site C, there was no relationship between the mean number of pollen grains/visit and time after anthesis at which the visit occurred (Figure 2).

The mean number of pollen grains transferred onto female phase flowers during anthesis was low in both countries, c. 5.9 and 4, at Sites B and C respectively. The number of pollen grains recorded on stigmas at Site C varied significantly between days ($P = 0.005$, unpaired t-test).

The mean number of pollen grains on the mesh covered control flowers at Site B and C was 0.29 and 0.20 respectively.

Proportion of insect visitors carrying avocado pollen

At Site A, 68.6% of the bees caught on female phase flowers were carrying pollen available for pollination, compared with 84% at Site C.

Bees collected from female phase flowers at Site A, with no pollinizer trees nearby, were carrying significantly fewer pollen grains (mean (\pm s.d.) 4.7 \pm 6.04) than bees caught at Site C (mean (\pm s.d.) 38.7 \pm 92.2) ($P = 0.01$, unpaired t-test).

Bees caught on male phase 'Bacon' flowers were carrying a mean (\pm s.d.) of 672 \pm 936 pollen grains.

Discussion

Our results show that in the two Australian avocado orchards there are a range of pollinators, including a variety of Diptera (most commonly hover flies), and Hymenoptera (in particular, European honey bees), and a species of stingless bee. The diversity of insects observed is consistent with the findings of Vithanage (1990). In New Zealand, the majority of pollinators observed (97.4%) were European honey bees. The only other potential pollinator recorded was a brown blowfly (*Calliphora stygia*).

In the Australian orchard, all the female phase flowers that were observed for their entire flowering period received multiple insect visits (mean visits/flower = 10). The New Zealand orchard had a lower mean number of visits/flower; 2.3, and of the observed flowers, 17.7% did not receive an insect visit. This suggests that part of the reason for less than optimal pollination in New Zealand may be low visitation rates. The data analysed for the Australian orchard were restricted to those collected on one day, because of poor weather. One day of good weather amongst multiple days of cold/wet weather may have affected the foraging behaviour of the insects. For this reason, we consider that our current Australian data are too sparse to determine the degree of visitation in this orchard.

We measured the pollen deposition on avocado stigmas to establish whether these insects were transferring avocado pollen. In Australia, all the visited female phase flowers observed for their full flowering period had pollen deposited on their stigmas. The mean number of pollen grains on these visited flowers was 5.9. In New Zealand, 68% of the visited flowers observed for their full flowering period had no pollen on them. A mean of four pollen grains was deposited on visited female phase flowers; however, this varied considerably between days. If avocado stigmas need at least 20 pollen grains to set fruit (Shoval 1987), our results suggest that the pollinating insects in the two orchards studied did not transfer enough pollen. This study did not observe the frequency of visits by individual insects and therefore we are not able to determine the effectiveness of the various insects as avocado pollinators.

Our study found that insects were carrying low numbers of avocado pollen grains when visiting female phase flowers, during a period where no overlap was evident between male and female flowers on the same tree or cultivar. In Australia, in a block with no pollinizers, 68.6% of the bees that were washed were carrying pollen (mean = 4.7 pollen grains/bee). The pollen found on these bees may be the result of foraging trips on male phase 'Hass' flowers the previous day. In New Zealand, in a block with pollinizers, 84% of the bees were carrying pollen (mean = 38.7 pollen grains/bee). The number of pollen grains found on honey bees in this study is much lower than those found by Vithanage (1990). However, Vithanage did not specify when his bees were caught; they may have been caught at a time when female and male flowers overlapped. Furthermore, his method did not state whether the bees were caught on male or female phase flowers. In this study, bees caught on male phase 'Bacon' flowers in the New Zealand orchard were carrying up to 2500 pollen grains, indicating that honey bees are capable of carrying large numbers of avocado pollen grains. The low pollen count suggests the bees on the female phase flowers had not recently visited a male phase flower and therefore had few pollen grains available to transfer to avocado stigmas.

In the Australian orchard, the mean number of pollen grains/insect visit increased when flowers were visited at least 3.2 hours after anthesis. This corresponds to the time at which flowers on the same tree and/or cultivar have reopened in their male phase (close pollination). This indicates that much of the pollination occurs in a short time frame, near the end of the female phase when the male phase flowers open. This is potentially problematic, because the timing and duration of both phases of flowering are affected by climatic conditions; thus, in unsuitable weather, this bisexual phase may not occur. This trend was not evident in the New Zealand orchard. The presence of pollinizer trees meant that cross-pollination could occur throughout anthesis. However, since there were a large number of visits recorded at this orchard that did not result in pollen being transferred, we suspect that bees were not frequently crossing between cultivars.

Both the low insect visitation (in New Zealand), and the low numbers of pollen grains transferred onto avocado flowers (at all sites), may be factors that are currently limiting pollination and consequently fruit set in some orchards in Australia and New Zealand. Further research in these countries should focus on determining the effectiveness of different insects at pollinating avocado flowers and investigating the overlap between male and female phase flowers (taking into account the timing of anther dehiscence), under various climatic conditions. If under current growing conditions there are times when not enough pollen is being transferred, then there is potential to better manage pollination of avocado, thus increasing fruit production in 'Off' years.

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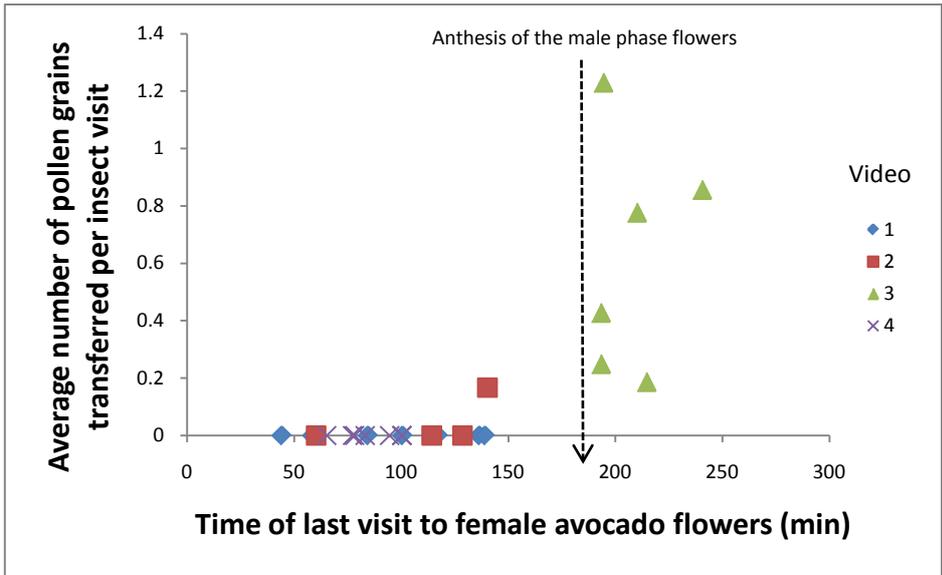


Figure 1. The mean number of pollen grains deposited onto individual avocado flowers per insect visit v. the time of the last recorded visit to each flower at Site B (Australia).

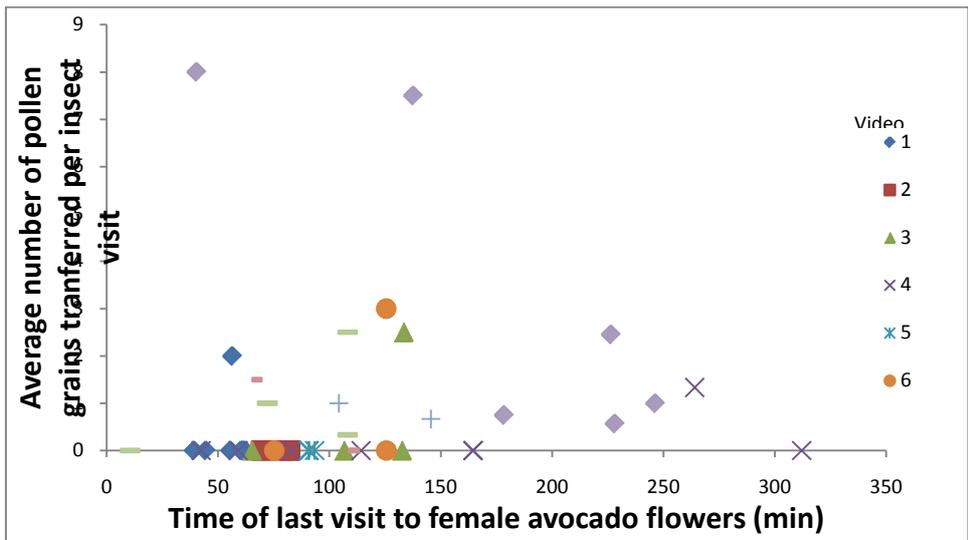


Figure 2. The mean number of pollen grains deposited onto individual avocado flowers per insect visit v. the time of the last recorded visit to each flower at Site C (New Zealand).

Table 1. The approximate times at which both the female and male phase avocado flowers opened and closed during the study period. The variation in flowering time is caused by changes in climatic conditions such as temperature.

| Site | B (Australia) | | C (New Zealand) | |
|------------------------|-------------------------|-----------------------|-------------------------|-----------------------|
| Date (Video number) | Female Hass anthesis | Male Hass anthesis | Female Hass anthesis | Male Hass anthesis |
| 26/10 (1 & 2) | 1200 - 1600 | 1500 – 1600+ | - | - |
| 27/10 (3 & 4) | 0730 - 1230 | 1015 – 1230+ | - | - |
| 31/10 (1 & 2) | - | - | 1600 - 1900 | 1900+ |
| 2/11 (3 & 4) | - | - | 1330-1730 | 1630-1730+ |
| 4/11 (5 & 6) | - | - | 1330-1700+ | 1630-1730+ |
| 8/11 (7& 8) | - | - | 1500-1800 | 1600-1800+ |
| 9/11 (9&10) | - | - | 1200-1700 | 1300-1800 |