THE CONTRIBUTION OF HONEY BEES, FLIES AND WASPS TO AVOCADO (PERSEA AMERICANA) POLLINATION IN SOUTHERN MEXICO

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Abstract—Although avocado is native to Mexico, there are no comparative measures in this country on the performance of its flower visitors as pollinators. The contribution of honey bees, flies and wasps to the pollination of avocado from tropical Mexico was assessed by comparing abundance, speed of flower visitation, quantity of pollen carried per individual and pollen deposited on virgin flowers after single visits. The values of abundance and frequency of flower visitation with pollen deposition were combined to obtain a measure of pollinator performance (PP). The most abundant insects on avocado were flies (mean ± SE: 15.2 ± 6.2), followed by honey bees (9.4 ± 6.3) and wasps (4.2 ± 3.1) (ANOVA F = 91.71, d.f. = 2.78; P < 0.001). Honey bees and wasps visited similar number of flowers (8.2 ± 3.1 and 7.5 ± 2.6 respectively), and more than flies (4.1 ± 1.2) in the same time period (F = 17.63; d.f. = 2.33; P < 0.01). Although flies carried far more avocado pollen on their bodies (44.9 ± 16.8 grains) compared with honey bees and wasps (21.3 ± 6.2 and 23.8 ± 8.11 grains, respectively; H = 26.522, df = 2, P = 0.001), the number of pollen grains deposited on a stigma after a single visit was similar for the three taxa (2-5). There was evidence for a significant and similarly positive PP of both honey bees and flies as avocado pollinators over wasps, given their abundance, potential for pollen transport and deposition of pollen on stigmas.

Keywords: pollinator, Hymenoptera, Diptera, pollen deposition, fruit set

INTRODUCTION

Avocado (Persea americana) is a tree of the family Lauraceae native to southern Mexico and Central America. In its native environment, an array of insect taxa visit avocado flowers, including bees, wasps (Hymenoptera) and flies (Diptera) (Free 1993; Castañeda-Vildózola et al. 1999; Can-Alonzo et al. 2005). However, the relative contribution of different insect taxa to avocado pollination has never been evaluated in its native Mexican range.

Avocado trees have bisexual flowers that show synchronously protogynous dichogamy (Free 1993). The flowers alternate between the female or pistillate stage I, and the male or staminate stage II on consecutive days. The flowers produce nectar in both stages (Davenport 1986). There are two types of avocado cultivars; those classified as type A have flowers in stage I in the morning of their first opening and then the same flowers re-open again in stage II of the following afternoon. Cultivars classified as type B have flowers in stage I in the afternoon of their first opening and stage II on the following morning (Lahav & Gazit 1991). Although there is still debate as to whether insects are needed for avocado pollination (Davenport 1998), it has been shown that insect visits significantly increase fruit set in most cultivars worldwide (Vithanage 1990; Ish-Am & Eisenkowitz 1998a).

In extensive avocado orchards, it is common practice to introduce honeybee colonies (Apis mellifera) to promote pollination. However, honey bees show erratic rates of visitation of avocado flowers and tend to reduce their visits when there are other blossoms in the surroundings, which may negatively affect fruit-set in the absence of other insect visitors (Ish-Am & Eisenkowitz 1993, 1998a; Ish-Am et al. 1999). It has even been suggested that honey bees do not visit avocado flowers assiduously because of the repellent properties of the high mineral concentrations and the alcohol perseitol in its nectar (Afik et al. 2006; Afik et al. 2011). Because avocado is native to the Neotropics, some of its floral traits could make it attractive to its natural pollinators but possibly less so for honey bees that are exotic to this geographic region (Ish-Am et al. 1999; Afik et al. 2011). Yet there is surprisingly little information on the behaviour of avocado’s insect visitors in Mexico and Central America, and practically no comparative measures of their efficiency as pollinators.

Mexico is the world’s largest producer of avocado with ca. 40% of the total production and avocado represents a major export crop for the country (Sánchez-Colín & Arriaga...
In Mexico, extensive avocado plantations are heavily sprayed and the surrounding forests have been largely logged, so the diversity and numbers of insects have been greatly reduced within plantations (Castañeda-Vildózola et al. 1999). Thus, it is important to evaluate the relative contribution of native insects to avocado pollination in order to propose appropriate and sustainable management programs for this economically important crop in Mexico. Ensuring the productivity of cash crops such as avocado is of particular importance in the tropics in order to improve the environmental performance of agriculture in these areas (Foley et al. 2011).

Measuring pollinator performance is difficult but such measures predominantly assess pollinator behaviour and estimates of stigmatic pollen deposition (Freitas & Paxton 1998; Ne’eman et al. 2010). In this study, the individual visitation activity of honey bees, flies and wasps was evaluated per unit time and their abundance on avocado flowers. The values of visitation and abundance were also combined with the quantity of pollen deposited on virgin flowers after a single visit to compare the relative performance of the different taxa for avocado pollination.

**MATERIALS AND METHODS**

The study was conducted in an avocado orchard in the locality of Hunucmá, 89° 99’ 29” N and 21° 07’59” W in the Yucatan Peninsula. The climate in this area is tropical with a range of precipitation between 800 and 1500 mm annually. This orchard held a total of 240 trees, mainly of the ‘West Indian’ race, planted in alternate rows of type A and type B, 12 trees per row. The age of the trees was on average 5 years. The orchard was routinely sprayed with insecticides and was surrounded by mango and citrus cultivars.

The experiments were conducted from February to April 2010 which encompass the flowering period of avocado in the Yucatan (Can-Alonzo et al. 2005). Insects were collected at flowers in three consecutive weeks and identified in the Laboratory of Entomology of the Campus of Biological Sciences in UADY. Wasp specimens belonged to the species *Beachygastra mellifica* and *Polybia occidentalis*. Flies were mainly of the species *Chrysomya megacephala* and *Osmia obesa* and a few *Musca* sp. In the analyses we only included individuals of *B. mellifica* and *C. megacephala* as these were the most abundant species in the wasp and fly taxa, respectively.

**Relative contribution of open and hand cross-pollination to avocado fruit set**

The relative contribution of insects to avocado pollination was estimated by comparing open pollination and hand cross-pollination versus zero insect visitation using bagging methods (Kearns & Inouye 1993; Dafni et al. 2005).

Twenty trees of type ‘A’ were selected for the experiments. Three panicles with only flower buds were selected at random from each of tree and covered with cheesecloth bags before anthesis. When the flowers started to open, a group of flowers on each of the three panicles were left unbagged across the experiment and their fruit set represented the effect of open pollination. Another group of flowers was cross-pollinated with two drops of a fresh solution obtained by macerating the anthers of 20 mature type B flowers in 1 ml of distilled water. The two drops contained an average of 800 pollen grains. A third group of flowers on each panicle remained bagged during the whole experiment and represented the effect of zero insect visitation. Flowers were marked with different coloured thread depending on the treatment that they received and fruit set was recorded 2 weeks after the experiments.

**Contribution of different insect taxa to avocado pollination**

This part of the study was conducted in March 2010, in order to carry the experiments during the flowering peak of both, type A and B trees. The relative contribution of three insect taxa to avocado pollination was evaluated using three approaches. During 5 min we counted the number of individuals of each taxon that were present on 100 female flowers of 10 type “A” trees at 8 am, 10 am and 12 pm in two occasions separated by one week. These data were used to calculate the average abundance of each taxon per min ($A$). For periods of 3 min, we observed 12 individuals per taxon to determine the average number of female flowers visited per min. An estimation of flower coverage ($FC$) was obtained multiplying the abundance of each insect type ($A$) by the number of flowers visited per individual per min ($NF$).

Thereafter, estimates of pollinator performance in picking up and transferring avocado pollen grains to avocado stigmas were obtained. Insects of the three taxa visiting flowers in phase I were collected and their body was washed twice using the protocol of Freitas (1997). The average number of avocado pollen grains per individual ($P$) was obtained by counting two aliquots of the washed off grains in a haemacytometer (Dafni et al. 2005). In the case of honey bees, the rear legs were removed before washing to avoid sampling of corbicular pollen loads. The avocado pollen grain has distinctive shape, form and size and thus, is easy to differentiate from those of other plants that were found on the bodies of the three insect types (see Appendix I). An index of the number of avocado pollen grains removed and transported per taxon, $PT$ (for ‘pollen transportation’), was then calculated as $PT = A \times P$. Three specimens from each taxon were also analysed under the microscope to determine the body regions on which pollen grains were more frequent.

Finally, the number of avocado pollen grains deposited ($PD$) on virgin flowers of type A in stage I by the three taxa of pollinator was recorded. For this, we used flowers from previously bagged inflorescences and attached them to a wire stick on the day that they first opened. The stick was then taken close to avocado trees for an insect to visit the flower (Appendices II and III). The stigmas of the visited flowers were fixed in Fuchsin-stained glycerine jelly and the avocado pollen grains attached to the stigma surface were counted under a microscope using the protocols by Kearns and Inouye (1993).
Pollinator performance

The parameters of flower coverage by each insect taxa and the number of grains deposited in a single visit were combined to obtain an estimate of pollinator performance (PP) (Olsen 1997; Ne’eman et al. 2010) as follows:

\[ PP = FC \times PD \]

Where:

\[ FC = \text{Flower coverage (a product of the abundance of a given taxon on flowers at stage I)} \]
\[ PD = \text{Number of pollen grains deposited per single visit.} \]

Statistical analyses

The abundance of each insect taxon was compared using a two-way ANOVA with tree and insect taxon as main effects on the log transformed data. In the case of numbers of flowers visited per individual, we compared insect taxa by means of a one-way ANOVA. For analysis of both abundance and number of flowers visited by insect taxa, we subsequently used Duncan’s multiple comparison tests to compare taxon means.

Numbers of avocado pollen grains carried by each insect taxon were compared by means of a Kruskal-Wallis test followed by a Dunn multiple comparison test. The number of pollen grains deposited on the stigma and the individual values of pollinator performance were also compared between insect taxa by means of Kruskal-Wallis and Dunn multiple comparison tests. All statistical analyses were conducted using the SAS statistical package 9.2 (SAS Institute Inc. 2008).

RESULTS

Relative contribution of open and cross-pollination to avocado fruit set

It was found that only a small number of flowers in the bagged inflorescences set fruit (Tab. 1). On the other hand, both the open and cross-pollination treatments had similar percentages of fruit set that were higher than the treatment with permanently bagged flowers (Tab. 1). However, as hand self-pollination trials were not performed, it was not possible to differentiate the relative contribution of self and cross-pollination on fruit set.

Contribution of different insect taxa to avocado pollination

The abundance of honey bees, flies and wasps at avocado flowers (A) differed significantly (F = 91.71, d.f. = 2,78; P < 0.001). The most abundant insects per 100 flowers in stage I of anthesis were flies (15.2 ± 6.2), followed by honey bees (9.4 ± 6.3) and wasps (4.2 ± 3.1).

Honey bees and wasps visited a similar number of flowers per min (NF = 8.2 ± 3.1 and 7.5 ± 2.6, respectively). However, flies visited on average only half the number of flowers visited by the other two taxa in the same period (Tab. 2) (F = 17.63; d.f. = 2,33; P < 0.001).

Significant differences between insect taxa in the number of avocado pollen grains that each insect type carried on its body were found (H = 26.522, df = 2, P = 0.001). On average, flies carried over twice the number of avocado pollen grains on their bodies compared with honey bees or wasps (Tab. 2). Therefore, flies, due to the large number of avocado pollen grains on their bodies compared to both honey bees and wasps and their high abundance on the crop, had the largest pollen transportation value (PT; Tab. 2).

Pollinator performance

Flowers on sticks were quickly visited once taken close to the trees, usually in less than 5 min. Pollen deposition on the stigma after a single visit (PD) showed no differences between insect taxa (H = 5.23, df = 2, P = 0.072; Tab 2). There were differences regarding the areas on which avocado pollen grains were observed on the body of each insect type. The areas on which pollen grains were more abundant on the honey bee were the first two pair of legs, the ventral part of the thorax and the last abdominal segment. Flies, in contrast, carried pollen mainly in the ventral region between their thorax and abdomen and the last two abdominal segments. In the case of wasps, pollen grains were present in the mandibles, the base of the first pair of legs and the ventral part of the thorax. Possibly all the body regions where pollen was found in the three insect taxa are good sites for transfer of pollen to stigmas as there were no differences in pollen deposition (Appendices I and II).

When the values of PP per individual were compared between the three taxa, no significant differences were detected between flies and honey bees, but wasps had lower values compared with the other two taxa (H = 9.533, df = 2, P = 0.01; Tab 2).

DISCUSSION

In a cultivated avocado orchard in southern Mexico we found that three major insect groups were attracted in significant numbers to flowers, namely non-native bees (A. mellifera) and flies (C. megacephala) and native wasps (B. mellifica). Similar values were found for the pollinator performance of both, honey bees and flies; but wasps were less important for avocado pollination on this particular site, possible a result of their lower numbers.
We also corroborated the idea that pollen vectors have a beneficial effect on fruit set of avocado cultivars in southern Mexico, as bagged flowers set fewer fruit. However, it is not possible to clearly establish whether it is self or cross-pollination that flower visitors undertake, as hand self-pollination trials to separate both were not performed. Yet other studies have demonstrated that autogamy in avocado flowers does not lead to fertilization (Free 1993; Ish-Am et al. 1999). Flies have been suggested to be potential pollinators in its native range (Vischer & Sherman 1998) that attract different insect taxa (Roubik et al. 2002). Flies have also been reported as frequent visitors of avocado flowers and they have been considered as potential pollinators in its native range (Vischer & Sherman 1998). On avocado flowers in tropical Yucatan, we also found that the fly *C. megacephala* was highly abundant and that the most frequent wasp was *B. mellifica* which indicate that they are present across the different regions where avocado is cultivated. These latter two species along with the honey bee visited both type A and B trees (pers. obs.) and had avocado pollen grains on body areas that may effectively contact the stigma. Indeed, the body regions on which the pollen was found on the honey bees significantly touch the stigma of the avocado flower (Ish-Am & Eisikowitch 1993), and the same could be true for flies and wasps.

It is interesting that none of the studied insects was best for all the indicators of pollinator ability that we evaluated. They did not carry equal amounts of avocado pollen, and they were not equally abundant nor visited similar number of flowers per unit time. For instance, honey bees were capable of visiting a larger number of flowers compared to flies and wasps. Their advantage with respect to flies was through the higher numbers of flowers that they visited per time unit and, with respect to wasps, it was through the higher density of honey bees. However, honey bees carried significantly less pollen on their bodies compared with flies. In contrast, flies were more abundant on flowers and seemed more capable of moving larger numbers of pollen grains, but they visited less flowers per unit time. Regardless these differences, the indices of pollen deposition (*PD*) on virgin stigmas were similar for all three taxa. Yet when the values of *PD* were combined with *FC*, it was evident that the honey bees and flies were equally important for avocado pollination and more important compared with wasps. Nevertheless, when considered together, flies and wasps could have had a wider *FC* and overall importance compared with the honey bees. Such a combined effect of flies and wasps is relevant if and when honey bees are attracted to nearby blossoms other than avocado.

Unlike most bee-pollinated flowers where floral rewards are usually hidden (Kevan and Baker 1983), avocado flowers have exposed nectar and easily collected pollen (Vischer and Sherman 1995; Castañeda-Vildózola et al. 1999). Moreover, the morphological features of avocado flowers do not seem to fit such a view that flies may have low pollinator efficiency in its natural environment.

The importance of Diptera as pollinators cannot be neglected. They are one of the most important groups of...
pollinating insects, second only to the Hymenoptera (Kearns 2001; Symank et al. 2008). Flies are regular visitors of at least 100 cultivated plant species including economically important crops (Heath 1982). Although flies are usually regarded as generalist flower visitors (Faegri and Van der Pijl 1979), they are present in many ecosystems and their role in pollen transfer could be considerable, mainly due to their large numbers (Symank et al. 2008), as our results show with Calliphoridae in Yucatecan avocado orchards.

The use of insecticides could have been an important factor that reduced the diversity of insects visiting avocado flowers in our study site. In non-commercially cultivated/sprayed orchards from other regions of Mexico, over 100 insect species have been documented visiting avocado (Ish-Am et al. 1999). We only found two species of wasp and 5 species of fly belonging to the families Calliphoridae (1 species) Syrphidae (2 species) and Tachinidae (2 species). Surprisingly, meliponine bees, which are considered good avocado pollinators (Castañeda-Vildózola et al. 1999; Can-Alonzo et al. 2005), were not seen in the orchard during our experiments possibly due to the continuous use of insecticides, too.

In the light of the erratic visits of honey bees to avocado flowers and to ensure the presence of alternative pollinators in orchards, we consider essential to reduce and carefully plan the use of insecticides especially during the blossom period. It is also important to preserve forest patches around avocado orchards that provide nesting sites and food to ensure long term populations of alternative pollinators.

In multiple pollinator systems, the relative importance of a given pollinator may change as its populations fluctuate spatially and temporarily (Thompson and Thompson 1992; Kearns 2001; Symank et al. 2008). Therefore, although our preliminary findings revealed a positive performance of insects other than the honey bee on avocado pollination in southern Mexico, it is still necessary to further evaluate the contribution and combined effects of various insect visitors over seasons and cultivars in order to ensure sustainable levels of fruit production of this economically important crop across Mexico.

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APPENDICES

Additional supporting information may be found in the online version of this article:

Appendix I. Avocado pollen grains and other types of pollen found on the body of the three insect taxa.

Appendix II. *Beachyoga mellifica* lateral (left) and ventral view (right). Two white avocado pollen grains can be seen on the base of the first left leg (white arrow).

Appendix III. *Chrysorynxa megacephala* (Diptera) (A); and honey bee (*Apis mellifera*) (B) visiting virgin avocado flowers on a stick.

REFERENCES


