CONTRIBUTION OF INSECT POLLINATION TO *MACADAMIA INTEGRIFOLIA, COFFEA ARABICA,* AND *DIMOCARPUS LONGAN* IN HAWAIʻI

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

ENTOMOLOGY

DECEMBER 2013

By

Jane Marion Tavares

Dissertation Committee:

Mark G. Wright, Chairperson
Ethel Villalobos
Don Drake

Keywords: *Apis mellifera,* insect pollinators, pollen transfer, fruit yield

ACKNOWLEDGMENTS

First of all I would like to thank the USDA-TSTAR research program for funding this project: Grant # 2010-34135-21499.

I would like to thank the Honeybee Lab for the opportunity to do my thesis on honeybees and learn about beekeeping in Hawaii.

I would like to thank Scott Nikaido for being a wonderful beekeeping teacher, and for his help. I would also like to thank Tyler Ito for his help and encouragement, but most importantly, to both Scott and Tyler for having such a great sense of humor which always kept me laughing.

To my committee, Dr. Mark Wright, Dr. Ethel Villalobos, and Dr. Don Drake for their help and expertise in macadamia, coffee and longan, honeybee pollination, and Botany.

To beekeepers Charlie Reppun and George Hudes for sharing their knowledge on beekeeping and teaching me how to do honey extraction.

To Charlie Reppun for the use of his coffee orchard and Wayne Ogasawara for the use of his longan orchard.

To Dr. Luc Leblanc for helping me to identify flies and for Dr. Glen Taniguchi for helping me to identify ants.

To Dr. Koon-Hui Wang for her support, and guidance. To both Dr. Koon-Hui Wang and Dr. Marbel Zapotica for their friendship and their positive encouragement.

I am forever thankful to my family for their constant support, both loving and occasionally financial, which without them the completion of this thesis would have been very difficult, if not impossible.

ABSTRACT

The contribution of insect pollination was assessed for three crops in Hawaii: *Macadamia integrifolia, Coffea arabica, and Dimocarpus longan.* These three plants, having very different mating systems, were shown to be visited by a range of insects in the Orders Hymenoptera, Diptera, Lepidoptera, and Coleoptera. While Diptera ranked highest in species richness, *Apis mellifera*, the honeybee, was the most abundant insect visiting the flowers in all three study orchards. Overall fruit set and fruit retained was increased with insect pollination, as well as fruit quality. Abundance, combined with foraging behavior and stigma contact suggested that honeybees were the greatest contributors to pollination for these crops.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
POLLINATION OF TROPICAL CROPS

1.1. Introduction

The Hawaiian archipelago is one of the most geographically isolated groups of islands in the world, and is home to a large number of endemic insects including a set of unique bee species in the genus *Hylaeus*. These endemic bee species are closely linked to native plants and seldom visit introduced plants (Daly and Magnacca, 2003). Although highly important from an ecological standpoint these species do not provide pollination services to farmers. As such, pollination services of crop species in Hawaii are highly dependent on a single introduced species: *Apis mellifera*, the European honeybee (Nagata, 2012). Macadamia nut (*Macadamia integrifolia*), coffee (*Coffea arabica*), and longan (*Dimocarpus longan*) are three crops considered to benefit from insect pollination, honeybees in particular. In Hawaii macadamia nut and coffee are of large economic importance and longan is among one of the many tropical fruit crops grown in Hawaii. The aims of this thesis are therefore; to determine species richness and abundance of the insect foragers, quantify impact of insect pollination on fruit set, yield, and fruit quality, and to estimate the pollen transfer efficacy of its major pollinators for macadamia and coffee. The following chapters cover each of the three plants; macadamia nut, coffee, and longan. This chapter outlines the pollination of tropical plants, importance of fruit crops in Hawaii and their dependency on bee visitation to achieve productivity, the importance of the honeybee in Hawaii, and introduces the three study plants.

1.2. Pollination of Tropical Plants

Pollination research is an important aspect in agriculture. Understanding the pollination requirements for crops allows us to determine the need for pollinator conservation, pollination management, and to help ensure the successfulness of the crop. The pollination of tropical plants can be specific in regards to their pollination needs (Roubik, 1995). For example, flower morphology can limit insect visitation, thus preventing certain insects from reaching the floral rewards; nectar or pollen and thereby

preventing those insects from pollinating the flower. The atemoya (*Annona atemoya*) flowers are such a flower. It will discourage large insects from visiting due to its narrow floral openings. The beetles from the family Nitidulidae, which are small, are able to reach both the receptive stigmas and mature pollen producing anthers of atemoya flowers, thus effectively pollinating the atemoya (Nagel et al., 1989). Although some tropical plants may require particular pollinators, many crops in tropical regions rely on non-specialized pollinators for fruit production, such as native stingless bees and managed or feral colonies of honeybees (*Apis mellifera*) (Roubik, 1995). Stingless bees such as *Trigona* and *Melipona* are two genera of social bees that form large colonies. They are known to have important pollination contributions for several crops (Heard, 1999). Hawaii lacks *Trigona* and *Melipona*; therefore, the honeybee is the main non-specialized pollinator of various crops in Hawaii.

1.3. Importance of Fruit Crops in Hawaii

Since the decline of the large sugar and pineapple industries, diversified agriculture has become important in Hawaii. Specialty tropical fruits (e.g. atemoya, cherimoya, lychee, persimmon, rambutan, starfruit, mango, and longan) have potential to become significant local industries for small-scale growers. In 2008 tropical specialty fruit totaled 1,480 acres in Hawaii, and the value of sales was $4.0 million during this year (Hudson et al., 2008). Hawaii grows an assortment of crops, both tropical, and temperate. A variety of these crops require insect pollination for adequate fruit set. Table 1.1 shows various crops and fruiting plants in Hawaii with emphasis on whether or not supporting research has shown honeybees to have an important role in the pollination of the crop. Often feral honeybees assist in providing pollination for these crops. Small scale beekeepers may also be providing pollination for these crops either intentionally or unintentionally. In some instances farmers with large orchards, particularly those with macadamia nut, will bring in large numbers of managed honeybee colonies to increase crop yield. Our dependency on honeybees in Hawaii is unquantified; however, honeybees have been in Hawaii for a moderate length of time and are known to be beneficial in crop pollination.

1.4. Importance of Honeybees in Hawaii

1.4.1. Honeybee History in Hawaii

Honeybees were successfully introduced to Hawaii in the mid 1800's, after a few failed attempts, and successfully established in 1857 (Greene, 1941). That year three hives of dark German bees *A. mellifera mellifera* were shipped from San Jose, California to Honolulu and once they had made it to Hawaii they were purchased by the Royal Hawaiian Agricultural Society (Greene, 1941; Nieman, 1942). These colonies eventually became established in the Nu'uanu Valley (Nieman, 1942). Since the introduction of honeybees to Oahu they have also been introduced to the Hawaiian Islands of Niihau, Kauai, Molokai, Lanai, Maui, Kahoolawe, and Hawaii (Roddy and Arita-Tsutsumi, 1997).

In the first decades following the introduction of the honeybee into Hawaii, honeybees existed solely in feral colonies or in hives managed by hobbyists. It wasn't till the late 1890's when beekeeping became more than just a hobby (Greene, 1941). It was at this time that cattle ranching began in the Hawaiian Islands and the kiawe (*Prosopis pallida*) tree became an important source of wood, shade, and fuel. The kiawe beans were also a source of feed for cattle. Honeybees were established near ranching operations on the Island of Hawaii to increase kiawe bean yield. Beekeepers soon found that the kiawe nectar produced an excellent honey which led to the commercial production of honey in Hawaii (Nieman, 1942; Greene, 1941). In 1881 the first macadamia nut trees were imported to Hawaii and were used as either an ornamental or for reforestation (CRFG, 1997). Hawaii's first macadamia nut farm was established in 1925. Eventually more orchards were planted and the grafting of macadamia nut cultivars led to mass production and wide scale planting (Takeguchi, 2009). The macadamia nut inflorescences were seen as an exceptional source of nectar and pollen for expanding honeybee colonies (Roddy and Arita-Tsutsumi, 1997). Early on it was common for Hawaiian macadamia nut growers to have honeybee apiaries in their orchards to facilitate cross-pollination and to increase crop yields (Nieman, 1942).

1.4.2. The Importance of Studying the Honeybee in Hawaii

Many studies have shown that honeybees increase crop yields, and are an important component in pollination. Therefore, honeybees are considered to be important for pollination in many crops for Hawaii. However, specific details on their importance relative to other potential pollinators have not been fully studied in Hawaii. To acquire this information it would be necessary to look at the abundance and richness of insects that visit the flowers of these crops, to analyze their effects on fruit set, and to analyze the foraging activities of the foragers. Another factor underscoring the need to understand the interactions that occur between pollinators and agriculture is the recent advent of honeybee pests into Hawaii, an area long free of severe honeybee parasites. The arrival of *Varroa destructor* (varroa mite) and *Aethina tumida* (small hive beetle) has caused a recent dramatic decline in Hawaii honeybees. As we determine ways to deal with these pests, through the use of organic treatments, non-organic treatments (when needed), and changing our beekeeping practices, managed hives will likely be restored; however, feral hives will not be provided such management, and are likely to be severely reduced in numbers. The effects from the loss of feral colonies that will occur on pollination are yet to be studied. A dearth of feral hives will hasten the need to have a clear understanding of the pollination contributions of honeybees. The information in this thesis will be important for Hawaii and to the farmers growing the following study crops, macadamia (Proteaceae) and coffee (Rubiaceae), and longan (Sapindaceae).

1.5. Introduction to the Family Proteaceae

The plant family Proteaceae comprises 79 genera and around 1,700 species. The family is mostly prevalent in the southern hemisphere, but some species occur in the northern hemisphere as well, but limited to Africa and south-east Asia. The center of diversity for the family Proteaceae is Australia, followed by southern Africa, temperate to tropical South and Central America, New Caledonia, Southeast Asia from India and Japan to Indonesia, New Guinea, Madagascar, Fiji, New Zealand, and Vanuatu (Smith et al., 2004). This family is comprised of evergreen trees and shrubs and is diverse in leaf morphology including leaves that can be broad, fern-like, or conifer-like. The flowers (individual florets) of this family are usually small and borne in dense inflorescences. The

number of flowers per inflorescence varies with species. There can be as many as 1000 flowers per inflorescence as seen with the *Banksia ericifolia* (Levin, 2008) or considerably less, only about 70 flowers per inflorescence like *Grevillea beadleana* (Smith and Gross, 2002). The flowers have a uniseriate perianth (Smith et al., 2004). This indicates that the perianth parts are in a single whorl around the stigma (Simpson, 2010). The perianth is comprised of four petal-like structures called tepals. They are referred to as tepals because all aspects of the perianth are similar in shape and color, having no distinguishing characteristics to differentiate between the petals and sepals. They have four stamens which are attached to the tepals. An interesting trait that is shared among most of the genera within this family is that their stigmas function as "pollen-presenters". This term is used because when the flower opens and the stigma becomes visible, the pollen, and in some cases the most easily accessible pollen for foragers, can be found directly on the stigma. Only a few genera in this family are the pollen foragers actually able to remove pollen directly from the anthers. The nectary glands are intrastaminal, located near the stamens, or extra floral [in some genera, e.g. *Leucospermum*]. Insect pollinators are the most frequent among Proteaceae with some species of Proteaceae being pollinated by birds (Smith et al., 2004). In Hawaii there are several non-native species of Proteaceae in the following genera; *Banksia, Grevillea, Leucadendron, Leucospermum, Protea,* at least one species of *Dryandra, Hakea, Isopogon, Petrophile* and *Telopea* (Starr and Starr, 1997); however, the one Proteaceae species most commonly associated with Hawaii is *Macadamia integrifolia;* the macadamia nut.

Macadamia integrifolia is indigenous to the eastern coast of the subtropical rainforests of Australia (Wallace et al., 1996). It was brought to Hawaii in 1881 to be used as an ornamental and for reforestation. In 1948, Hawaii researchers produced several varieties of *M. integrifolia* which led to the development of a successful macadamia nut industry (CRFG, 1997). Many aspects of the macadamia tree have been studied by researchers to help improve and maintain nut production and quality in their area.

1.5.1. Flower Biology for *Macadamia integrifolia*

Florets (100-300) are borne on inflorescences (Trueman and Turnbull, 1994b). The flowers are white, fragrant and hermaphroditic; however, they lack petals, but have petaloid sepals instead (Figure 1.1). Petaloid sepals are modified sepals that look like petals. Macadamia has the "pollen presenter" characteristic as previously mentioned (Figure 1.1). When the stigma emerges from the perianth it is covered in pollen. Research has shown that the stigma becomes fully receptive at two days after flower has opened; however, maximum pollen germination and pollen tube growth occurs when flower is three days old. The delay in stigma receptivity allows for insect foragers to remove pollen while foraging, thus increasing chances for out-crossing (Sedgley et al. 1985). The flowers are protandrous in that the anthers mature and shed pollen before the stigma is receptive. Protandry is often seen in insect pollinated plants (Willmer, 2011). Interestingly, the macadamia is considered to be partially self-compatible, meaning that it can produce fruit by self-pollination; however, the degree of self-compatibility depends on the cultivar (CRFG, 1997; Sedgley et al., 1990).

The macadamia stigma is capable of accepting 10 to 12 pollen grains for pollination (Luce, 1977). Once a flower is pollinated, the pollen grains will germinate as soon as the stigma becomes receptive (Sedgley et al., 1985). A few days later, the petals will shrivel up and fall off leaving behind the stigma, style, and ovary, and around seven days later the ovary will become slightly swollen (Ito et al., 1970). Within the first few weeks following pollination, it is common for the majority of the florets to be aborted from the inflorescence. The remaining fruits continue to increase in size up to 18 weeks after flowering. Gradual loss of macadamia nuts will continue till 28-30 weeks after flowering (Sakai and Nagao, 1985). The harvest of macadamia nuts begins around six to nine months after fruit set, which is indicated by the mature nuts falling from the trees (Heard, 1993; Wallace et al. 1996). It is important to note that not all florets that initiate fruit development will become harvestable nuts. Most inflorescences will begin with 100 to 300 florets (Trueman and Turnbull, 1994b); however, the average raceme will retain a very small percentage of fruits. One reason why plants will produce so many flowers is to attract lots of pollinators (Nattero et al., 2011). This could be the reason for the numerous flowers produced per inflorescence in macadamia. Approximately 90% of the initial fruit

set will be aborted (Sakai and Nagao, 1985). Reasons why abortion of fruit set occurs include insufficient pollination, reducing crop load to avoid depletion of storage carbohydrates needed for fruit development, or unfavorable climatic conditions (Trueman and Turnbull, 1994b; Stephenson et al., 1989; Stephenson and Gallagher, 1987*).* Because the trees tend to progressively lose fruits over the first four months following pollination, studies will report fruit retentions and yields as fruit developments. However, while tracking the fruit set is an integral part for pollination studies, it is also necessary to record pollinators seen foraging while the macadamia orchard is in bloom.

1.5.2. The Contribution of Insect Pollination to the Macadamia Inflorescence

Insect pollinators are important for macadamia nuts, as they tend to aid in pollination (Heard, 1993). In Australia the native pollinator, *Trigona* sp. (Apidae) is seen frequently foraging on macadamia blooms, and has been observed to have a positive impact on macadamia pollination (Heard and Exley, 1994). Macadamia nut inflorescences have been observed to be attractive to a number of insect visitors including flies, bees, beetles, moths, and butterflies (Heard and Exley, 1994; Wallace et al., 1996). Species richness and abundance was studied among 15 orchards on the east coast of Australia. The insect orders recorded in their orchards were Hemiptera, Coleoptera, Diptera, Lepidoptera, and Hymenoptera; equaling a total of 55 species of insects. Social insects, *A. mellifera*, and *Trigona carbonaria*, were the most abundant. All other insects seen visiting macadamia flowers contributed less than one percent of the flower visitors. *Apis mellifera* and *T. carbonaria* were the only two insects seen consistently visiting macadamia nut flowers among all of their orchard sites, and therefore, were considered to be important pollinators (Heard and Exley, 1994). In Hawaii there have been no studies of macadamia nuts involving species richness or abundance, although field observations have noted that along with honeybees, syrphid flies are also known to visit macadamia flowers in Hawaii (Shigeura, 1967). Besides identification and quantifying individuals visiting flowers, it is also important to observe the insects foraging behavior. In a three year study in Australia, researchers observed the pollen foraging by both the honeybee and *Trigona*. It was determined that due to their constant contact with the stigma while foraging for pollen, these two bees' pollen foraging behavior was beneficial for

pollinating macadamia inflorescences (Wallace et al., 1996). In preliminary observations some insects were considered not to be effective in cross-pollination for macadamia because they were small, rarely observed visiting flowers, and were able to avoid contact with the stigma and the anthers while foraging for nectar (Heard and Exley, 1994). Along with insect abundance, it appears that foraging behavior of an insect is also an important factor and can influence an insect's impact on macadamia pollination and fruit set.

As mentioned earlier, macadamia trees abort fruits over the first four months after flowering. The most common time intervals used to facilitate comparisons across studies are 10-14 days post-anthesis, 21 days post-anthesis, and at harvest (Wallace et al., 1996; Sedgle et al., 1990; Ito and Hamilton, 1969). A final count can also be done at four months post-anthesis, at which time nuts are no longer being aborted. There are a number of studies in Australia that have compared the fruit set of inflorescences that had insect visitation, or insects excluded (spontaneous self-pollination). The impact on fruit production of insect pollination versus spontaneous self-pollination has been studied primarily in Australia. One study, by way of hand- pollination, compared the initial fruit set of cross-pollination to self-pollination of Hawaiian cultivars and found that cross-pollination produced more fruits compared to inflorescences that were self-pollinated (Ito and Hamilton, 1969). The studies from Australia and Hawaii have an overall commonality. They showed that at the stages in which the fruit set was recorded, inflorescences that had insects excluded commonly produced a lower amount of fruits compared to inflorescences that were either insect pollinated or were cross-pollinated by hand. Comparisons of nut weights have shown that inflorescences that were cross-pollinated by hand did increase nut weight. It was concluded that to increase nut quality it is important to augment the movement of pollen by way of outcrossing (Wallace et al., 1996; Trueman and Turnbull, 1994a).

The potential of a pollinator to transfer pollen can be determined by how many pollen grains the pollinator deposits onto the stigma. Research in Illinois, which observed bird and insect pollinators that visit trumpet creeper, found that the pollinator that deposited the most pollen, having the most contact with the stigma, was the more efficient pollinator, and also resulted in the highest fruit set. These pollen grain counts also supported the field observations of the pollinator's behavior relative to flower

structure (Bertin, 1982). Another study in Colorado was carried out to compare pollen efficacy of the foragers visiting *Penstemon* by how much pollen was removed from flower's anthers and how much pollen was deposited onto the stigma (Castellanos et al., 2003). By recording pollen transfer, both of these studies were able to quantify the efficacy of the main pollinators seen in the orchards; therefore, pollen transfer is an essential aspect to include when carrying out pollination studies.

1.6. Introduction to the Family Rubiaceae

The plant family Rubiaceae comprises around 650 genera and 13,000 species. The family is found worldwide, but is mainly pantropical. This family consists mostly of trees, shrubs, and some herbs. The leaves are simple (undivided blade), and entire (smooth margins). The overall corolla shape for this family is sympetalous meaning that petals form a tube or tubular shape (Ukers, 1922; Smith et al., 2004). The number of stamens usually equal the number of corolla lobes. The flowers can be borne singly as seen in gardenias or in clusters like *Ixora* and *Pentas* (Smith et al., 2004). Hawaii has several native species belonging to this family, 61 endemic and 2 indigenous species. The native species in Hawaii include *Bobea* spp., *Gardenia* spp., *Gynochthodes trimera, Psydrax odorata*, *Nertera granadensis, Coprosma spp., Kadua spp.,* and *Psychotria spp.* (Starr and Starr, 1997; Wagner et al., 1999). Some well-known plants of Rubiaceae in Hawaii are gardenias and *Morinda citrifolia*, commonly known as noni; however, one Rubiaceae that is an important commodity to Hawaii is the coffee plant *Coffea arabica*.

Coffea arabica is indigenous to Ethiopia where it grows in the moist highland sub-tropical forests in the Kaffa region of Western Ethiopia. Different cultivars of coffee can be found across eastern and tropical Africa; however, the '*arabica*' variety originated in Kaffa (Martins, 2008). The coffee variety '*arabica*' made its way to Arabia from Ethiopia, and around 1700 it was introduced into Indonesia by the Dutch. By 1706 a small number of plants made their way to Amsterdam. A few seedlings from Amsterdam were brought to Surinam, and from there they made their way to Brazil in 1727 (Griffin, 2006; Sybenga, 1960). Coffee was first brought into Hawaii from Brazil in 1825, but it wasn't till the 1930's when it became a fully established crop consisting in over 1,000

farms in Kona. Today coffee plants can be found on Hawaii's five main islands (Hawaii Coffee Association, 2001).

1.6.1. Flower Biology of *Coffea arabica*

Flowers are borne in clusters of two to 20 in the leaf axils, are white, fragrant, and hermaphroditic in that they have both pistil (female part) and stamens (male part), and have five stamens attached to the inside of the corolla (Figure 1.2). Nectaries can be found at the base of the corolla. Once the flower opens the pollen is shed and the stigma is receptive (McGregor, 1976). A stigma that hasn't received pollen can stay receptive from a few to nine days depending on humidity and temperature (Sybenga, 1960; Clifford and Willson, 1985). Once a flower is pollinated, the pollen grains will germinate, and pollen tube growth begins (Mendes, 1941). The corolla will wilt, and shrivel up in one to two days after pollination has occurred (Clifford and Willson, 1985). Soon the flower will fall off, leaving behind the ovary. The ovary will begin to swell and develop over several months. The harvest of coffee fruits begins around seven to nine months after pollination (Clifford and Willson, 1985; Vergara and Badano, 2009). Twelve to 20 fruits per node are considered very good fruit retention. Loss of fruit can be due to drought, nutrient deficiency, or defoliation (Clifford and Willson, 1985). As fruit reaches maturity the fruit will turn red and/or will be slightly soft when squeezed. This will indicate that the fruit is ready to be harvested (Personal communication from coffee grower).

Coffea arabica is self-compatible, meaning that it can obtain fruit by self-pollination; however, it has been shown in studies that insect visitation among coffee plants can increase fruit set (Raw and Free, 1977; Klein, et al., 2003a,b). The coffee flower has features that promote autogamy. The anthers and stigma are similar in height, and the anthers release viable pollen when stigma is receptive. However, there is a physiological mechanism that promotes cross-pollination. Research has shown that pollen tube growth on the stigma is faster if the pollen grain is from another coffee plant compared to pollen from the same plant (Mendes, 1961). Fast pollen tube growth can be important for different reasons. In zucchini faster tube growth resulted in a higher success rate of fertilization of ovules, thus improving fruit and seed quality (Davis et al., 1987). Fast pollen tube growth can also be important when weather conditions shorten the

longevity of the pistil thereby insuring fertilization of the ovule (Fakhim Rezaie et al., 2011). Another possibility that promotes cross-pollination is that *C. arabica* may be amphicarpic meaning that some flowers need cross-pollination while other flowers are able to develop fruits by self-pollination (Raw and Free, 1977; Roubik, 1995). To promote the benefits of outcrossing in coffee, insect pollinators can be a great contributor.

1.6.2. The Contribution of Insect Pollination to the Coffee Flower

There is little information regarding the types of insects visiting coffee flowers in its native habitat, in the Kaffa region of Ethiopia; however, information has been collected regarding insect visitation in the Jimma region of Ethiopia. Insects in the order Hymenoptera were the most frequent visitors to the coffee flowers. The insects observed included two honeybees, *Apis mellifera scutellata*, *Apis mellifera monticola*, a carpenter bee, *Xylocopa sp.*, and several unidentified solitary native bees. Two diurnal hawkmoths, *Cephonodes hylas* and a *Macroglossum* sp., were also observed to a lesser degree foraging on coffee flowers (Martins, 2008). Studies done in coffee orchards in Indonesia, Jamaica and in Mexico found that the common insect orders seen were Hymenoptera, Lepidoptera, Diptera, and Coleoptera (Klein et al., 2003b; Raw and Free, 1977; Vergara and Badano, 2009). *Apis mellifera* was the insect in highest abundance visiting coffee flowers among the orchards in the studies done in Jamaica and Mexico, and therefore, considered to be important for coffee pollination (Raw and Free, 1977; Vergara and Badano, 2009). The range of species richness varied among locations; 15 species in Mexico, 18 species in Jamaica, and 29 species in Indonesia (Klein et al., 2003b; Raw and Free, 1977; Vergara and Badano, 2009). Although insects pollinators are considered to be important pollinators in coffee (Roubik, 1995), ants may also contribute to pollination. Philpott et al. showed that branches exposed to both flying pollinators and ants had higher fruit weights then branches that were exposed to either flying pollinators or bagged to exclude insects (2006).

Despite *C. arabica*'s ability to be self-compatible the benefits of insect pollination within coffee orchards have been considered. Previous studies have recorded fruit sets at distinct time intervals. The most common time intervals used to facilitate comparisons

across studies are within the first three months after flowering and at harvest (Manrique and Thimann, 2002; Roubik, 2002; Vergara and Badano, 2009; Klein et al., 2003a). Studies in Jamaica, and Indonesia compared the fruit set of flowers that had insect visitation, insects excluded, and/or various hand-pollination treatments. Results between these treatments had an overall commonality. They showed that insect excluded flowers produced a lower amount of fruits compared to flowers that were either insect pollinated or were hand-pollinated. In Jamaica it was concluded that coffee plants caged with honeybees had 52% more fruits at harvest than plants completely excluded from insects (Raw and Free, 1977). In Indonesia is was concluded that insect pollinated flowers had 27% more fruit at harvest than flowers excluded from insects, and geitonogamy (flowers pollinated from pollen from another flower of the same plant) had 9.6% more fruit than flowers excluded from insects (Klein et al., 2003a). Comparisons of bean weights have shown that flowers exposed to insects had a significant increase in bean weight (Manrique and Thimann, 2002; Raw and Free, 1977). It was concluded that to increase bean yield and/or quality it is important to augment the movement of pollen throughout orchard (Klein et al., 2003a; Manrique and Thimann, 2002; and Raw and Free, 1977).

Another aspect of consideration for bean quality is the formation of peaberries. A coffee fruit usually contains two beans. In some instances only one bean will be found in a coffee fruit. When this occurs these beans are referred to as peaberries. The normal percentage of peaberries to occur is 10-15% (Clifford and Willson, 1985). The result of peaberries can be due to caging of coffee plants (Raw and Free, 1977), poor pollination, seasonal influences, or meiotic irregularities (Sybenga, 1960). While seasonality and genetics cannot be controlled or altered in an existing orchard; improving pollination would be the easiest way to reduce peaberries.

The potential of a pollinator to transfer pollen can be determined by how many pollen grains the pollinator deposits onto the stigma. Research in Costa Rica observed stigmas that were exposed to insect pollinators after 24 hours relative to distance from a nearby forest. At 50 meters they found about eight bee visits occurred on 100 flowers for 20 minutes and that at this distance about 1170 pollen grains were transferred on to coffee stigmas over 24 hours. At further distances from the forest the amount of pollen grains found on stigmas reduced significantly. It was concluded that calculating pollen

deposition was a very informative way to measure pollinator activity (Ricketts, 2004). The amount of pollen transferred onto stigmas is an important factor. Not enough pollen grains on stigma can result in a decrease in fruit growth, fruit maturation, yield and seed quality (Lee, 1988; Björkman, 1995).

1.7. Introduction to the Family Sapindaceae

The plant family Sapindaceae comprises 147 genera and 2,215 species. The family is mostly tropical and subtropical. The center of diversity for Sapindaceae is tropical America and tropical Asia. This family consists of trees, shrubs, and lianas (woody vines). Leaves are usually alternate but can be opposite, and are deciduous or persistent (Smith et al., 2004). The flowers are generally small in this family and plants can be dioecious (having male and female parts) or monoecious (having either pistil or stamens but not both) (Steentoft, 1988). Bees are considered to be very important pollinators for this family, however, birds and wind are also known to facilitate in pollination (Smith et al., 2004). In Hawaii there are several native species of Sapindaceae including three endemic species, *Alectryon macrococcus* var. *auwahiensis*, *A. macrococcus* var. *macrococcus* and *Sapindus oahuensis* and two indigenous species, *Dodonaea viscosa*, and *Sapindus saponaria* (Starr and Starr, 1997, Wagner et al., 1999). Some well-known plants of Sapindaceae in Hawaii, due to their fruit, are *Nephelium lappaceum* (rambutan), *Litchi chinensis* (lychee), and *Dimocarpus longan* (longan), our study plant.

Dimocarpus longan is native to southern China, in the provinces of Kwangtung, Kwangsi, Schezwan, and Fukien (Morton, 1987). Longan was introduced into Hawaii from China in 1852 (Tin-Yuke and Char, 1975). Today Hawaii has its own longan varieties, ‘Kohala’ and ‘Egami’, and continues to grow several varieties of longan from China and Thailand (Wood, 2004).

1.7.1. Flower Biology for *Dimocarpus longan*

Flowers are small, yellow-brown and borne on panicles up to 30 cm long that have around 3400 flowers per panicle (Menzel and Waite, 2005; Nakata and Sugiyama,

2005). Longan has three types of flowers. Flowering in each panicle progresses as staminate flowers (Type I, having stamens but lacking pistils), functionally female flowers (Type II, having pistils) (Figure 1.3), and functionally male flowers (Type III, having stamens). Flower types overlap each other during bloom. Type I flowers open first followed by Type II then Type III. The entire bloom can last about four to six weeks. The ratio of male to female flowers can be 5:1 or 6:1 (Menzel and Waite, 2005; Kongpitak et al., 1986). Cool winter temperatures are needed to induce flowering, so in tropical regions flowering can be erratic (Yuon, 2007). In Hawaii applications of potassium chlorate solution in soil is used to force plants to flower (Nagao and Ho-a, 2000).

Once a flower is pollinated, fertilization occurs in about 42 hours (Xinghui et al., 1996). Female flowers are bicarpellate (having two fruits). However, usually only one locule in each flower will develop. Fruits will take 120-150 days to mature, each panicle usually having 80 individual fruits, and each fruits weighing five to 20 grams. Premium fruit will normally weigh 14-18 grams (Menzel and Waite, 2005).

1.7.2. The Contribution of Insect Pollination to the Longan Panicle

Insect pollinators are important for longan, as they tend to augment pollen transfer from male flowers to female flowers, a necessity for fruit production of this plant (Menzel and Waite, 2005), whether it be between trees or within a single panicle (Blanche et al., 2006). Although honeybees and *Trigona* spp. are known to be important pollinators of longan, hoverflies are also known to visit longan flowers (Menzel and Waite, 2005; Boonithee, 1991, Blanche et al., 2006). Wind can also play a role in pollination of longan to some extent; however, to obtain high yields insects are considered to be the major contributor (Menzel and Waite, 2005; Blanche et al., 2006).

Previous studies have recorded the impact of insect pollinators on longan by recording fruit loss. Honeybees have shown to increase yields to 30% or even 90% (Manning, 2006). In Australia it was shown that panicles exposed to insect pollinators had 62% more fruit compared to panicles excluded from insects (Blanche et al., 2006). A study in Thailand showed that longan trees pollinated by insects resulted in a yield of about 20kg/tree, whereas trees caged from insects resulted in a yield of about 0.6 kg/tree

(Kongpitak et al., 1986). Similar to the other two study plants, insect pollinators can help to improve yield, and quality.

Overall, there have been limited studies in Hawaii involving macadamia, coffee, or longan. Specific objectives of this thesis are to 1) determine richness and abundance of the insect flower visitors, 2) quantify impacts of insect pollination on fruit set, retention and bean quality, and 3) estimate pollen transfer efficacy of key insect pollinators. It is anticipated that insect pollination in Hawaii could increase fruit set and retention in the study plants.

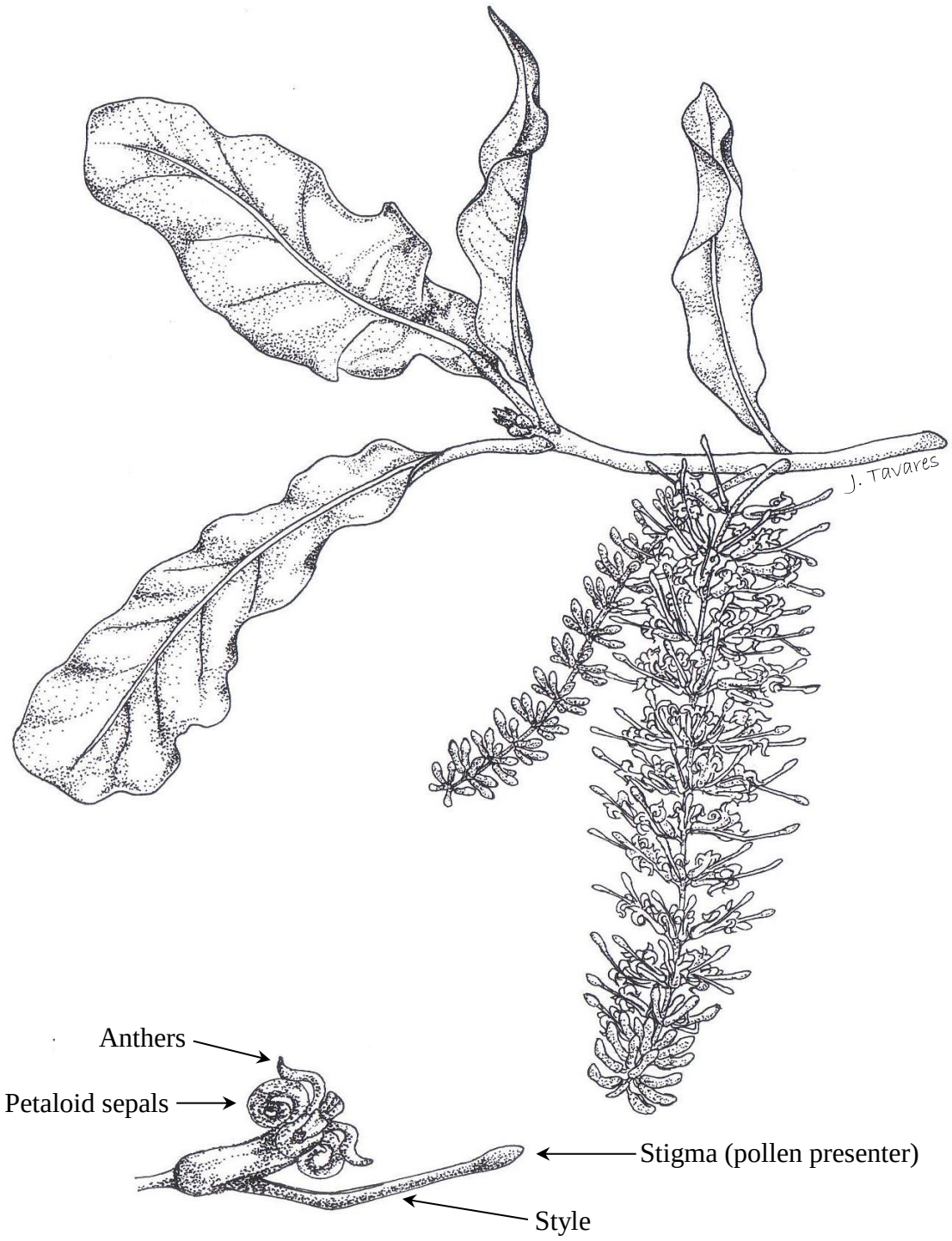

Figure 1.1. Drawing of a conflorescence (bunch) of inflorescences and a close-up of a single macadamia flower.

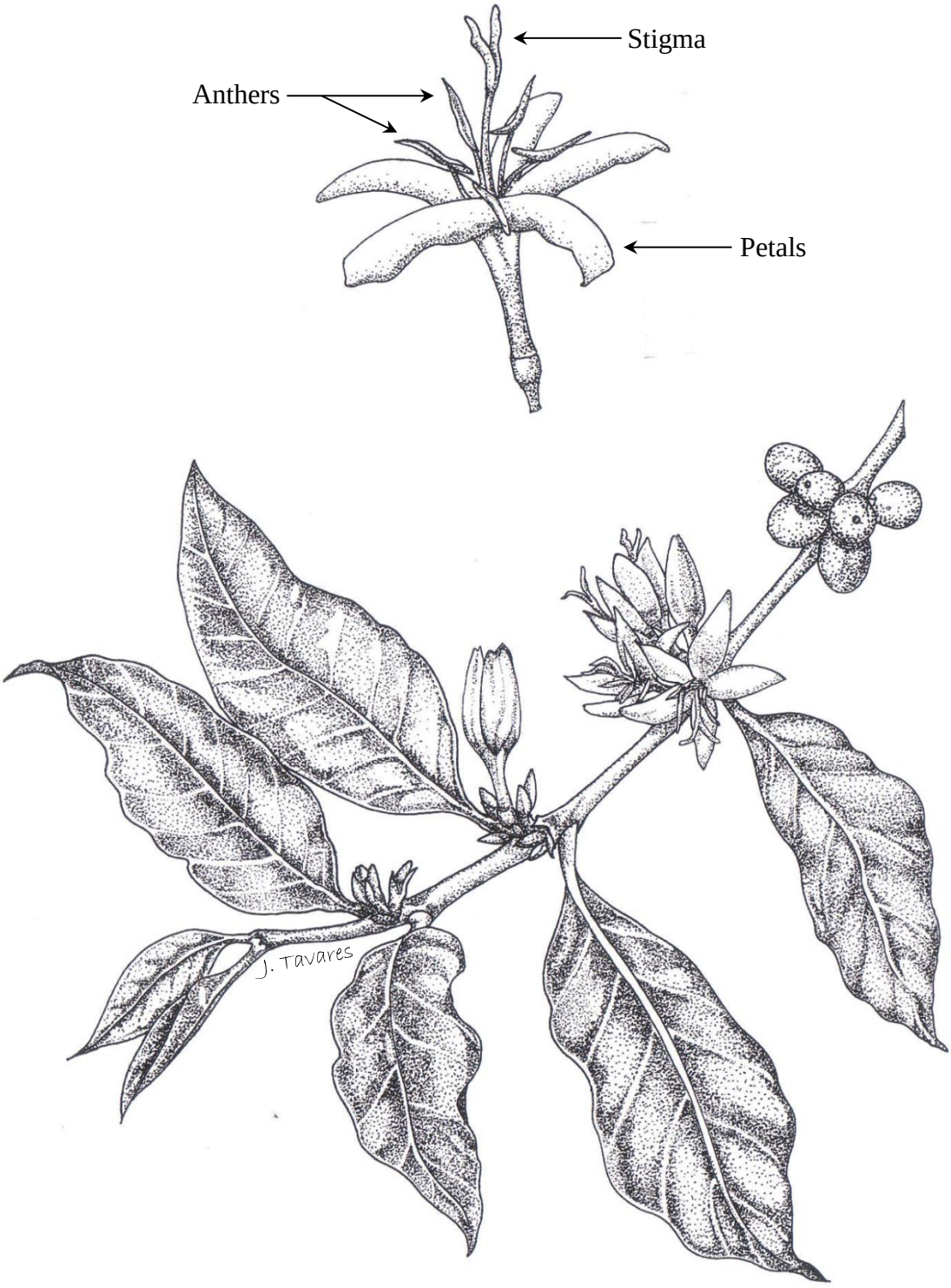

Figure 1.2. Drawing of a single coffee flower and a coffee branch showing nodes a different ages: budding, flowering, and fruiting.

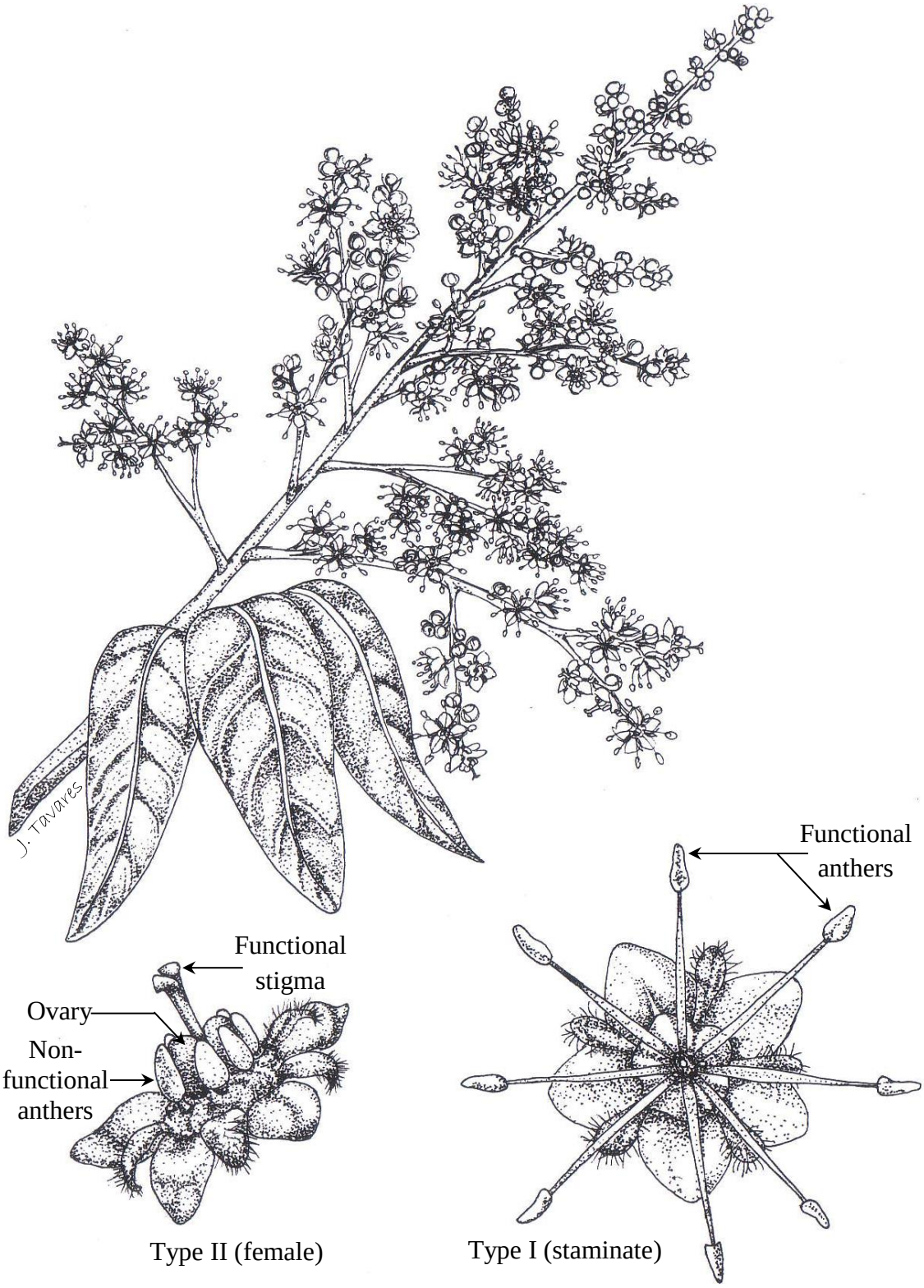

Figure 1.3. Drawing of a longan panicle and close-ups of the Type I and II flowers.

Table 1.1 Crops of Hawaii with emphasis on the honeybee's role as a pollinator. Insects in 'Associated Pollinator' column indicate insects known to increase fruit set, increase yield, and/or improve quality of fruit.

Crop	Associated Pollinator	Notes	Reference
Atemoya (*Annona atemoya*)	Nitidulidae: beetles	-	(Nagel et al., 1989)
Avocado (*Persea american*)	Honeybee and other insects	Dependent on insect pollination	(McGregor S. E., 2009; Keogh, et al., 2010a)
Blueberry (Southern Highbush) (*Vaccinium* spp.)	Honeybee and other insects	Depends on cultivar	(Morse and Calderone, 2000; Rhodes, 2006)
Cantaloupe (*Cucumis melo*)	Honeybee	Highly dependent on honeybees	(McGregor and Todd, 1952)
Cherry Surinam (*Eugenia uniflora*)	Unknown	Honeybees and carpenter bees known to visit	(NTBG, 2012)
Citrus (*Citru* spp.)	Honeybee	Honeybees are used for citrus that need pollination	(Sanford, 2003)
Coffee (*Coffea arabica*)	Honeybee and other insects	Pollinators beneficial for both self-sterile and self-fertile species	(Klein et al., 2003a)
Corn (sweet) (*Zea mays* var. *saccharata*)	None	Honeybees will forage for pollen	(Adams and Bartholomew, 2004)

Table 1.1 Continued

Crop	Associated Pollinator	Notes	Reference
Eggplant (*Solanum melongena*)	Honeybee and other insects	Self-pollinated but needs insects to achieve this, bees improve fruit set	(Al-abbadi, 2009)
Longan (*Dimocarpus longan*)	Honeybee and other insects	Pollination by wind may also be a factor	(Davenport and Stern, 2005, Keogh et al., 2010b)
Lychee (*Litchi chinensis*)	Honeybee and other insects	Pollination by wind may also be a factor	(Davenport and Stern, 2005; McGregor, 2009; Keogh et al., 2010b)
Mango (*Mangifera indica*)	Honeybee and other insects	Insects transfer pollen from anther to stigma within inflorescence or between cultivars	(Keogh et al., 2010c)
Mountain Apple (*Syzygium malaccense*)	Honeybees visit this plant in Hawaii (E. Villalobos personal observation)	-	-
Noni (*Morinda citrifolia*)	Honeybees	Capable of self and cross-pollination	(Lomnes, 2006; Nelson C. S., 2003)

Table 1.1Continued

Crop	Associated Pollinator	Notes	Reference
Pepper (*Capsicum* spp.)	Honeybee and other insects	Mostly self-pollinating, bees improve yield and quality	(Al-abbadi, 2009)
Persimmon (Maru) (*Diospyros virginiana*)	None	*Parthenocarpic; **however, insect pollination will remove tannins that cause astringency in the fruit	(Chia et al., 1989)
Pumpkin (*Cucurbita maxima*)	Honeybee and other insects	Bees known to be important to pollination	(Julier and Roulston, 2009)
Rambutan (*Nephelium lappaceum*)	Honeybee and other insects	Cross-Pollination necessary	(Erickson and Atmowidjojo, 2001)
Strawberry (*Fragaria ananassa*)	Honeybee and other insects	Self-fertile; however, pollinators improve quality of fruit. Honeybees: very efficient in pollinating	(McGregor S. E., 2009; Keogh, 2012d)
Starfruit (*Averrhoa carambola*)	Honeybee and other insects	-	(Callahan, 2010)

Table 1.1 Continued

Crop	Associated Pollinator	Notes	Reference
Tomato (*Solanum lycopersicum*)	None	Self-pollinating. Pollination can occur by gravity or vibration caused by insect or wind	(Delaplane and Mayer, 2000)
Watermelon (*Citrullus lanatus*)	Honeybee and other insects	Honeybees increases yield, even in seedless watermelons	(Stanghellini et al., 1998; Morse and Calderone, 2000)
Beans (*Phaseolus* spp.)	None	Self-pollinating, Honeybees can aid in pollination.	(McGregor S. E., 2009)
Lettuce (*Lactuca sativa*)	None	Self-pollinating, except for male-sterile plants in which case insect pollinators may be beneficial	(McGregor S. E., 2009)
Onion (*Allium* spp.)	Honeybees and other insects	Cross-pollination and seed production aided by insects.	(Manning, 2006; McGregor S. E., 2009)
Macadamia (*Macadamia integrifolia*)	Honeybee and other insects	Honeybees and hoverflies frequent foragers	(Morse and Calderone, 2000; Shigeura, 1967)

*Parthenocarpy is the ability to produce fruit without fertilization (Roubik, 1995).

** 'Maru' is a pollination-variant nonastringent variety which means fruits are edible when firm only if they have been pollinated. These fruits are astringent when seedless, resulting from un-pollinated flowers (CRFG, 1996).

1.8. References

Adams, R. G., and C. Bartholomew. 2004. Protecting honey bees from pesticide poisoning. State of Connecticut. http://www.ct.gov/deep/lib/deep/pesticide_ certification/Supervisor/protecting_honeybees.pdf.

Al-abbadi, S. Y. 2009. Efficiency of different pollination treatments on Solanaceae yields grown in plastic house. Journal of Biological Sciences. 9: 464-469.

Bertin, R. I. 1982. Floral biology, hummingbird pollination and fruit production of trumpet creeper (*Campsis radicans*, Bignoniaceae). American Journal of Botany. 69: 122-134.

Björkman, T. 1995. The effect of pollen load and pollen grain competition on fertilization success and progeny performance in *Fagopyrum esculentum*. Euphytica. 83: 47-52.

Blanche, R. K., J. A. Ludwig, and S. A. Cunningham. 2006. Proximity to rainforest enhances pollination and fruit set in orchards. Journal of Applied Ecology. 43: 1182-1187.

Boonithee, A., N. Juntawong, H. Pechhacker, and E. Hüttinger. 1991. Floral visits to selected crops by four *Apis* species and *Trigona* sp. in Thailand. Acta Horticulturae. 288: 74-80.

Callahan, J. R. 2010. Emerging biological threats: A reference guide. Greenwood Press, Santa Barbara, CA.

Castellanos, M. C., P. Wilson, and J. D. Thomson. 2003. Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. Evolution. 57: 2742-2752.

Chia, C. L., C. S. Hashimoto, D. O. Evans. 1989. General crop information: Persimmon. knowledge master. http://www.extento.hawaii.edu/kbase/crop/crops/i_ persim.htm.

Chia, C. L., and R. M. Manshardt. 2001. Why some papaya plants fail to fruit. Fruits and Nuts. College of Tropical Agriculture and Human Resources Extention publication F&N-5, Honolulu, HI.

(CRFG) California Rare Fruit Growers. 1996. Persimmon. http://www.crfg.org/pubs/ff/persimmon.html.

(CRFG) California Rare Fruit Growers. 1997. Macadamia. 1997. http://www.crfg.org/pubs/ff/macadamia.html.

Clifford, M., and K. Willson. 1985. Coffee botany, biochemistry and production of beans and beverage. AVI Publishing Company, Inc. Westport, CT.

Daly, H. V., and K. N. Magnacca. 2003. Insects of Hawaii Volume 17: Hawaiian Hylaeus (Nesoprosopis) Bees (Hymenoptera: Apoidea). University of Hawaii Press, Honolulu.

Davenport, T. L., and R. A. Stern. 2005. Flowering, pp. 87-113. *In* C. M. Menzel and G. K. Waite, Litchi and Longan: Botany, Production and Uses. CAB International, United Kingdom.

Davis, L. E., A. G.Stephenson, and J. A. Winsor. 1987. Pollen competition improves performance and reproductive output of the common zucchini squash under field conditions. Journal of American Society for Horticultural Science. 112: 712-716.

Delaplane, K. S., and D. F. Mayer. 2000. Crop pollination by bees. New York: CAB International, United Kingdom.

Erickson, E. H., and A. H. Atmowidjojo. 2001. Rambutan. *In* S. E. McGregor (ed.), Insect pollination of cultivated crop. http://afrsweb.usda.gov/sp2userfiles/place/53420300/ onlinepollinationhandbook.pdf.

Fakhim Rezaie, Sh., J. Hajilou, and F. Nahandi. 2011. Pollen germination and pistil performance in several Iranian peach cultivars. International Journal of AgriScience. 1: 170-177.

Greene, G. (1941). Honey in Hawaii. Paradise in the Pacific. p. 29.

Griffin, M. 2006. Brazilian coffee beans. Coffee Research Institute. http://www.coffee research.org/coffee/brazil.htm.

Hawaii Coffee Association. 2001. Coffee history in Hawaii. http://www.hawaiicoffee assoc.org/History.

Heard, T. A. 1993. Pollinator requirements and flowering patterns of *Macadamia integrifolia*. Australian Journal of Botany. 41: 491-497.

Heard, T. A. (1999). The role of stingless bees in crop pollination. Annual Review of Entomology, 44: 183-206.

Heard, T. A., and E. M. Exley. 1994. Diversity, abundance, and distribution of insect visitors to macadamia flowers . Environmental Entomology. 23: 91-100.

Hudson, M. E., K. Whetstone, N. Morita, and K. Lee. 2008. Hawaii tropical specialty fruits. USDA-National Agricultural Statistics Service. http://www.nass.usda.gov/ Statistics_by_State/Hawaii/Publications/Fruits_and_Nuts/tropfrt.pdf.

Ito, P.J., R. A. Hamilton. 1969. Initial nut set from cross- and self-pollination of six macadamia cultivars. Hawaii macadamia producers association annual proceedings. 9: 31-35.

Ito P. J., J. E. Hunter, and R. A. Hamilton. 1970. Effect of cross-pollination on initial and final nut set and yields of macadamia cultivars. Hawaii macadamia producers association annual proceedings 10: 16-19.

Julier, H. E., and T. H. Roulston. 2009. Wild bee abundance and pollination service in cultivated pumpkins: Farm management, nesting behavior and landscape effects. Journal of Economic Entomology. 102: 563-573.

Keogh, R., A. Robinson, and Mullins, I. 2010a. Pollination aware case study 4: Avocado. RIRDC. Australian Government Rural Industries Research and Development Corporation. Publication No. 10/081.

Keogh, R., A. Robinson, and Mullins, I. 2010b. Pollination aware case study 18: Lychee and longan. RIRDC. Australian Government Rural Industries Research and Development Corporation. Publication No. 10/125.

Keogh, R., A. Robinson, and Mullins, I. 2010c. Pollination aware case study 20: Mango. RIRDC. Australian Government Rural Industries Research and Development Corporation. Publication No. 10/127.

Keogh, R., A. Robinson, and Mullins, I. 2012d. Pollination aware case study 32: Strawberry. RIRDC. Australian Government Rural Industries Research and Development Corporation. Publication No. 10/139.

Klein, A.-M., I. Steffan-Dewenter, and T. Tscharntke. 2003a. Bee pollination and fruit set of *Coffea arabica* and *C. canephora* (Rubiaceae). American Journal of Botany. 90: 153-157.

Klein, A.-M., Steffan-Dewenter, I., and Tscharntke, T. 2003b. Fruit set of highland coffee increases with the diversity of pollinating bees. Proceedings of the Royal Society of London. 270: 955-961.

Kongpitak, P., P. Akratanakul, and S. Malaiphan. 1986. Study on pollination of longan by using honey bees as insect pollinator. Bangkok, Thailand.

Lee, T. D. 1988. Patterns of fruit and seed production, pp. 179-202. *In* E. B. Doust, and L. L. Doust, Plant reproductive ecology: Patterns and strategies. Oxford University Press, Inc., NY.

Levin, H. 2008. The Evolution of Proteaceae, in flower and leaf. Florida Wildflowers. http://www.flwildflowers.com/proteaceae/.

Lomnes, S. 2006. *orinda citrifolia*- The noni. http://bioweb.uwlax.edu/bio203/2011/lomnes_sydn/classification.htm

Luce, A. 1977. Honeybees and macadamia. Proceedings, Hawaii Macadamia Producers Association, 17th Annual Meeting. CTAHR. Kona, HI. pp. 52-57.

Manning, R. 2006. Bee pollination benefits for tropical fruit crops. Department of Agriculture and Food: Government of Western Australia. http://www.agric.wa.gov.au.

Manrique, A. J., and R. E. Thimann. 2002. Coffee (Coffee arabica) Pollination with Africanized Honeybees in Venezuela. Interciencia. 27: 414-416.

Martins, D. 2008. Coffee (Coffea arabica) in forest and agro-forestry cultivation in Jimma, Ethiopia. Initial survey of good pollination practices. Food and Agriculture Organization (FAO). Rome, Italy.

McGregor, S. E. 2009. Insect pollination of cultivated crop plants. http://afrsweb.usda.gov/sp2userfiles/place/53420300/onlinepollinationhandbook.pdf.

McGregor, S. E., and F. E. Todd. 1952. Cantaloup production with honey bees. Journal of Economic Entomology. 45: 43-47.

Mendes, T. A. 1961. Speed of pollen tube penetration in coffea arabica. Bragantia, 20: 495-502.

Mendes, A. J. T. 1941. Cytological observations in *Coffea*. VI. embryo and endosperm development in *Coffea arabica* L. American Journal of Botany. 28: 784-789.

Menzel, C., and G. K. Waite. 2005. Litchi and longan: botany, cultivation and uses. CABI Publishing, Cambridge, MA.

Morse, R. A., and N. W. Calderone. 2000. The Value of honey bees as pollinators of U.S. crops in 2000. Bee Culture Magazine, pp. 1-15.

Morton, J. 1987. Fruits of warm climates. Florida Flair Books, Miami, FL.

Nagao, M. A., and E. B. Ho-a. 2000. Stimulating longan flowering in Hawaii with Potassium chlorate. Journal of Hawaiian Pacific Agriculture. 11: 23-27.

Nagata, R. T. 2012. Honey Bees, kiawe and ranching intertwined. Hawaiian Tribune Herald. Stephens Media. Honoulu, HI.

Nagel, J., J. E. Pena, and D. Habeck. 1989. Insect pollination of atemoya in Florida. Florida Entomologist. 72: 207-211.

Nakata, M.; and N. Sugiyama. 2005. Morphological study of the structure and development of longan inflorescence. Journa of the America Society for Horticultural Science. 130: 793-797.

Nattero, J., Malerba, R., Medel, R., and Cocucci, A. 2011. Factors affecting pollinator movement and plant fitness in a specialized pollination system. Plant Systematics and Evolution. 296: 77-85.

Nelson, C. S. 2003. *Morinda citrifolia* L. Honolulu: Permanent Agriculture Resources, Holualoa, HI.

Nieman, R. E. 1942. Liquid Gold. Paradise of the Pacific. 17-21.

Nishina, M., F. Zee, R. Ebesu, A. Arakaki, R. Hamasaki, S. Fukuda, N. Nagata, C. L. Chia, W. Nishijima, R. Mau, and R. Uchida. 2000. Papaya Production in Hawaii. *Fruits and Nuts*. College of Tropical Agriculture and Human Resources Extention publication F&N-3, Honolulu, HI.

(NTBG) National Tropical Botanical Garden. 2012. Meet the Plants. http://ntbg.org/ plants /plant_details.php?plantid=5067.

Philpott, S. M., S. Uno, and J. Maldonado. 2006. The importance of ants and high-shade management to coffee pollination and fruit weight in Chiapas, Mexico. Biodiversity and Conservation. 15: 487-501.

Raw, A., and J. B. Free. 1977. The pollination of coffee (*Coffea arabica*) by honeybees. Tropical Agriculture. 54: 365-370.

Rhodes, J. 2006. NSW Department of Primary Industries. http://www.dpi.nsw.gov.au/__ data/assets/pdf_file/0017/84410/pf157-honeybee-pollination-of-blueberries.pdf.

Ricketts, T. H. 2004. Tropical Forest Fragments Enhance Pollinator Activity in Nearby Coffee Crops. Conservation Biology. 18: 1262-1271.

Roddy, K. M., and L. Arita-Tsutsumi. 1997. A history of honey bees in the Hawaiian Islands. Journal of Hawaiian and Pacific Agriculture. 59-70.

Roubik, D. W. 1995. Pollination of cultivated plants in the tropics, FAO Agricultural Services Bulletin 118. Food and Agriculture Organization (FAO). Rome, Italy.

Roubik, D. W. 2002. Feral african bees augment neotropical coffee yeild, pp. 255-266. *In* P. Kevan and V. L. Imperatriz Fonseca (eds.), Pollinating Bees - The Conservation Link Between Agriculture and Nature.Ministry of Environment, Brasilia.

Sakai, W. S., and M. A. Nagao. 1985. Fruit growth and abscission in *Macadamia integrifolia*. Physiologia Plantarum. 64: 455-460.

Sanford, M. T. 2003. Pollination of citrus by honey bees. University of Florida IFAS Extension. http://edis.ifas.ufl.edu/pdffiles/AA/AA09200.pdf

Sedgley, M., F.D.H. Bell, D. Bell, C. W. Winks, S. J. Pattison, T. W. Hancock. 1990. Self- and cross-compatibility of macadamia cultivars. Journal of Horticultural Science. 65: 205-213.

Sedgley M., M. A. Blesing, and H. I. M. V. Vithanage. 1985. A developmental study of the structure and pollen receptivity of the macadamia pistil in relation to protandry and self incompatibility. Botanical Gazette. 146: 6-14.

Shigeura, G. T. 1967. Varietal nut set and a suggestion of pollination requirement in macadamia, pp. 28-32. *In* Proceedins, 7th Annual Meeting Hawaii Macadamia Producers Association, May 1967, CTAHR. Kona, HI.

Simpson, M. G. 2010. Plant Systematics. Elsevier Inc. Burlington, VT.

Smith, J. A., and C. L. Gross. 2002. The pollination ecology of *Grevillea beadleana* McGillivary, and endangered shrub from northern New South Wales, Australia. Annals of Botany. 89: 97-108.

Smith, N., S. A. Mori, A. Henderson, D. W. Stevenson, and S. V. Heald. 2004. Flowering Plants of the Neotropics. Princeton University Press. Princeton, NJ.

Stanghellini, M. S., J. T. Ambrose, and J. R. Schultheis. 1998. Seed productino in watermelon: A comparison between two commercially available pollinators. HortScience. 33: 28-30.

Starr, F and K. Starr. 1997. Plants of Hawaii. http://www.starrenvironmental.com/images/?o=plants.

Steentoft, Margaret. 1988. Flowering plants in West Africa. Cambridge University Press, United Kingdom.

Stephenson, R. A. and E. C. Gallagher. 1987. Effects of temperature, tree water status and relative humidity on premature nut drop from macadamia. Scientia Horticulturae. 33: 113-121.

Stephenson, R. A., E. C. Gallagher, and T. S. Rasmussen. 1989. Effects of growth manipulation on carbohydrate reserves of macadamia trees. Scientia Horticulturae. 40: 227-235.

Sybenga, J. 1960. Genetics and cytology of coffee: a literature review. Bibliographia Genetica. 19: 217-316.

Takeguchi, A. E. 2009. History of agriculture in Hawaii. Department of Agriculture of Hawaii. http://hawaii.gov/hdoa/ag-resources/history.

Tin-Yuke, and Char, W. J. 1975. The first Chinese contract laborers in Hawaii, 1852. pp. 128-134.

Trueman, S. J., and C. G. N. Turnbull 1994a. Effects of cross pollination and flower removal on fruit set in macadamia. Annals of Botany. 73: 23-32.

Trueman, S. J., and C. G. N. Turnbull. 1994b. Fruit set, abscission and dry matter accumulation on girdled branches of macadamia. Annals of Botany. 74: 667-674.

Ukers, W. H. 1922. All about coffee. The Tea and Coffee Trade Journal Company. NY.

Vergara, C. H., and E. I. Badano. 2009. Pollinatior diversity increases fruit production in Mexican coffee plantations: The importance of rustic management systems. Agriculture, Ecosystems and Environment. 129: 117-123.

Wallace, H. M., V. Vithanage, and E. M. Exley. 1996. The effects of supplementary pollination on nut set of macadamia (Proteaceae). Annals of Botany. 78: 765-773.

Wagner, W. L., D. R. Herbst, N. Khan, and T. Flynn. 1999. Flora of the Hawaian islands. Smithsonian: National Museum of Natural History. http://botany.si.edu/pacificislandbiodiversity/hawaiianflora/query2.cfm

Willmer, P. 2011. Pollination and floral ecology. Princeton University Press. Princeton, NJ.

Wood, M. 2004. New options for lychee and longan fans and farmers. Agricultural Research. pp. 20-22.

Xinghui, L., Q. Dongliang, X. Chuanlong, and H. Liupeng. 1996. A study on the pollination biology of longan. CNKI: South China Fruits.

Yuon, L. C. 2007. Flowering induction of longan trees (*Dimocarpus longan* Lour.) grown in Sarawak. Agriculture research center, Semongok.

CHAPTER 2
CONTRIBUTION OF INSECT POLLINATION TO *MACADAMIA INTEGRIFOLIA* PRODUCTION IN HAWAII

2.1. Abstract

Apis mellifera (the honeybee) is accepted to be an important pollinator in many agricultural crop systems in Hawaii. However, specific details on the importance of *A. mellifera*, along with other insect visitors have not been determined for macadamia nut orchards in Hawaii. Reductions in feral honeybee populations in Hawaii attributable to invasions by varroa mites (*Varroa destructor*) and small hive beetle (*Aethina tumida*) have resulted in growers becoming dependent on managed bees, requiring increased understanding of the role they play as pollinators. Several aspects determining the contributions of insect pollinators in macadamia nuts were measured: 1) species richness and abundance of insects visiting macadamia flowers, 2) the effects of insect pollination in regards to fruit set, fruit retention, fruit size, and weight, and 3) insect pollen removal efficacy based on the mean number of pollen grains an individual insect removed from the stigma while foraging on a macadamia flower. The results from data collected in the study orchard concluded that while the order Diptera ranked highest in species richness (9 species), *A. mellifera* was the most abundant species visiting the macadamia inflorescences (62.7% in abundance, with an average of 17 honeybees seen per 15 minutes compared to 8 flies per 15 minutes). Inflorescences that were accessible to insects for pollination produced higher fruit sets and yield compared to inflorescences from which insect visitation was excluded. Abundance, coupled with foraging behavior and stigma contact suggested that honeybees are the greatest contributors to macadamia nut pollination over other insects observed in the orchard. The hoverflies observed in the orchard were shown to contribute to pollination as a whole, but to a lesser extent than the honeybee due to their low abundance.

2.2. Introduction

The macadamia nut (*Macadamia integrifolia*, Proteaceae) was ranked amongst Hawaii's top agricultural commodities in 2011(USDA, 2012), producing $38.2 mil farm gate value in 2011 (NASS, 2012). The macadamia nut industry is among the few agricultural industries in Hawaii to have formed a connection between farmers and the local beekeepers. The growers of large macadamia orchards have relied on beekeepers to bring in managed bee hives for pollination services. Despite the history of putative mutual benefits between the grower and beekeeper the importance of honeybees to the macadamia is unquantified. In the family Proteaceae, pollination may be complex, with various pollinators being important for different species, e.g. insects, birds, rodents and wind may all be significant vectors of Proteaceae pollination (Rourke and Delbert, 1977; Hattingh and Giliomee, 1989; Coetzee and Giliomee, 1985; Wright et al., 1991; Roubik, 1995), and it is thus important to quantify the relative importance of insects in macadamia nuts.

The recent onslaught of honeybee pests previously absent from Hawaii (*Varroa destructor* (varroa mite) and *Aethina tumida* (small hive beetle)) have reduced feral and managed bee colonies dramatically. There are no previous studies of the density of insect pollinators required to provide effective fruit set in macadamia nut orchards, as feral bees could simply augment pollination by managed hives. Understanding the importance of honeybees in macadamia orchards is underscored in the presence of the recent invasive impacts on bee colonies.

Previous research on macadamia nuts has highlighted several important aspects regarding pollination. Macadamia flowers are protandrous; a biological feature that is often seen in insect pollinated plants (Willmer, 2011), and thus consistent with macadamia nut being entomophilous. Quantifying insect abundance and species richness in macadamia orchards is therefore an important aspect to address in macadamia pollination (Heard and Exley, 1994). Comparing fruit set and yields from inflorescences visited by insects to insect-excluded inflorescences can show the importance of insect pollination in macadamia nuts (Wallace et al., 1996). Macadamia nut fruit quality (mean kernel weight) has been shown to improve with cross-pollination by hand (Wallace et al., 1996; Trueman and Turnbull, 1994), and is therefore another important aspect regarding

pollen transfer. The movement of pollen among plants is another key factor, especially with crops that need or benefit from cross-pollination.

It is important to take several factors into consideration to determine pollination efficacy of an insect, such as the amount of pollen transferred onto stigmas, and insect behavior when foraging on flowers (Abe et al., 2011; Wallace et al., 1996). However, the macadamia stigma is covered in pollen when it opens. To improve chances of cross-pollination it is necessary that the pollen be removed from a newly emerged stigma. Therefore, an insect's efficacy to remove pollen from a stigma is important to assess. This chapter focuses on evaluating the contribution of insect pollination on macadamia nuts in Hawaii. Specific objectives of this study were to examine: 1) species richness and abundance of potential pollinators visiting macadamia flowers, 2) the impacts of insect pollination on macadamia fruit quantity and quality, and 3) the pollen removal efficacy of insects while foraging on macadamia flowers.

2.3. Materials and Methods

2.3.1. Study Site

This study was conducted at the University of Hawaii Waimanalo Research Station (21˚33' N 157˚71' W) on the island of Oahu, between February 2010 and January of 2011 in Year I, and between March 2011 and March 2012 in Year II. The study orchard was relatively small (155 x 74 m^2), consisting of 91 macadamia nut trees in seven rows consisting of cultivars Kau 344 and Keaau 660. As is typical of commercial orchards, the two varieties are interspersed to promote cross-pollination. In the study plot the cv Keaau 660 plants were dispersed as every third tree within every third row (Figure 2.1). The 'Kau 344' cultivar was used for all experiments, with the exception of quantifying species richness and abundance, in which both cultivars were included. Fifty managed beehives were located on the northeast side of the orchard, between trees in the first and second rows.

2.3.2. Monitoring Insect Foragers for Species Richness and Abundance

Monitoring of insect foragers was conducted on clear sunny days, between 6:00 am and 5:00 pm when trees were in bloom in both seasons. Assessment of species richness was done through collection and identification of insects observed foraging on macadamia inflorescences. Insects were identified to species and a reference list compiled. Voucher specimens were deposited in the University of Hawaii Insect Museum. In addition to species richness of all flower visitors, insect abundance was also recorded. Insects were identified after Year I, so only honeybee abundance was recorded in Year I between February 26 and March 19, 2010. Insect abundance of each species was recorded in Year II between March 15 and March 24, 2011. In both years, the orchard began blooming in January and finished in April. Objectives 1 and 3 were conducted in February and March, when the orchard was in full bloom.

Only inflorescences that were attractive to insects were included in the abundance analysis. This included inflorescences one day before opening, and those with open flowers (Figure 2.2 B and C). Before individual macadamia florets open, the bud turns completely white and the style emerges from the perianth, creating a loop with the style (Figure 2. 3A). Since the stigma is still enclosed at this time, pollen from that particular floret is unavailable to insects; however, insects are able to reach the nectar at the base of the florets by inserting their mouth parts into the slit in the perianth, which was created by the emerging style. The flower opens by the next day making pollen available to insect foragers (Figure 2.3. B). On the second day of flowering, florets remain attractive to insects, still producing nectar (Figure 2.3 C), but by day three petals darken to tan (Figure 2.3 D) becoming less attractive to insects. By day 4 petals turn brown and will soon fall off (Figure 2.3 E).

During observations, notes were made on whether the insect being observed was actively collecting pollen or foraging on the flower's nectar. Determining whether a honeybee was foraging for pollen or nectar was based on the presence of pollen on the pollen baskets and the foraging location of the bee, coupled with the position of the pollen bearing structures in each flower. With flies, on the other hand, the determination of pollen or nectar foraging was made on the foraging location of the insect alone.

2.3.3. Transect and Focal Insect Counts

Two different methods were used to assess insect abundance: 1) transect insect count, where insects seen on inflorescences were recorded while the observer walked down the orchard rows, and 2) focal insect count, where individual inflorescences were watched uninterruptedly for 20 minutes and all insects seen visiting the inflorescence were recorded. Table 2.1 shows how these data were collected.

A fixed route was walked for the transect (Figure 2.1) and repeated, at most, three times in a continuous loop. All trees in the orchard were included in the study except those located right next to a hive or trees surrounded by tall weeds. The route was walked at a constant pace (average of 100 seconds between plants) monitoring inflorescences that were about 2.1 meters above the ground. About 40 inflorescences were observed within the 100 seconds. During transect counts; insects were classified as foraging for pollen, nectar, or both.

During the focal insect observations, an individual inflorescence was watched for 20 minutes. For these observations, inflorescences up to 2.6m above ground were observed using a ladder. A total of 30 inflorescences were observed for this analysis. Inflorescences often occur in bunches (conflorescences) and within these bunches, multiple inflorescences can be blooming simultaneously. To ensure that each inflorescence was equally attractive to insects, conflorescences that had only a single inflorescence blooming in a bunch were used for this analysis. During focal counts, insects were recorded on whether they foraged for pollen or nectar. Insects were also recorded on whether they foraged on flowers that had only the style emerging from the perianth (only nectar accessible) or from flowers that were completely open (pollen and nectar accessible).

2.3.4. Fruit Set and Fruit Retention

To assess the impact of insect visitation on macadamia nut production in both years, inflorescences at bud stage (Figure 2.2A) averaging 3.2 meters above the ground were used. These branches were either 1) bagged with a fine mesh cloth bag (about 625 holes per sq. inch, measuring about 71.1cm x 54.6cm) covering about 45.7 cm of branch, encompassing the conflorescence, and secured with a wire tied, which constituted the

"insect exclusion" (IE) treatment; or 2) tagged with flagging tape, but not bagged during the flower receptive stage, which constituted the "open pollination" (OP) treatment. Inflorescences for both years were selected based on several requirements: 1) booming at least a month after trees had begun blooming, when trees were in full bloom, and 2) located at the edge of the canopies of the trees. Inflorescences are often in conflorescences, so in Year II, branches with 1-4 inflorescences within a cluster were used.

Fruit retention was quantified. In Year I, three inflorescences per tree, with a total of eight trees, were measured to nearest millimeter to obtain the average length of an inflorescence. The number of florets per inflorescences was also recorded for each inflorescence. These measurements were taken to determine the variability in inflorescence length and number of florets per inflorescence.

Fruit set and fruit retention on each experimental inflorescence was recorded throughout the development period, from fertilization to harvest (eight months for Year I and seven months for Year II). Each treatment was allocated to each tree used in the study. To avoid any effects of bagging on initial fruit development, OP inflorescences were also bagged once flowers were no longer attractive to insects in Year II. Four weeks after bagging (amount of time for all inflorescences in the conflorescence to bloom and no longer be attractive to pollinators), the mesh bags were removed from all branches. For Year I, the sample size was 14 branches for IE (total of 38 inflorescences), and 10 branches for OP (total of 44 inflorescences). For Year II, the sample size was 40 branches for IE (total of 70 inflorescences), and 35 branches for OP (total of 63 inflorescences). Initially 40 branches per treatment were used in Year II, but during the study 5 branches in OP were lost due to accidental pruning of trees.

For Year I, fruit retention was recorded twice a week and at three main points during their development; 14 days after opening, 21 days after opening, and when fruits were no longer being aborted. For Year II, fruit retention was recorded for each inflorescence every three weeks and at three instances during their development (similar to Year I) until nuts were no longer being lost. One month prior to harvest, racemes were re-bagged to catch nuts as they fell. Bags were checked once a week to collect any fallen nuts. All nuts harvested were accumulated as total yield.

2.3.5. Nut Quality

Nut quality was assessed by recording length, width, and mass of individual nuts at harvest. Nuts were dried at 38°C for seven days in an incubator. Two measurements per fruit including length and width of the fruits and nuts were made to the nearest 0.01 mm using digital calipers. The measurements included width and length of 1) shell, and kernel, with the husk removed, and 2) individual kernels (Figure 2.4A, and B). Mass was measured to the nearest 0.01g using a digital scale. Fresh wet mass was measured for 1) weight of shell, and kernel, and 2) weight of individual kernels. For Year II, nuts were dried then weighed for final dry-weight values.

2.3.6. Efficacy of Insect Pollen Removal

Potential insect efficacy to remove pollen from the stigma was determined by quantifying the amount of pollen an insect removed from the pollen presenter (or stigma). As discussed in Chapter 1, the stigma of the macadamia flower is referred to as a pollen presenter. When the stigma emerges it is covered in pollen which then can be easily collected by an insect. Numbers of pollen grains on the stigma of newly open flowers were counted: 1) after one insect visit, 2) after floret was exposed for a whole day to insect visitation, or 3) with no insect visitation. To determine the amount of pollen removed by insect visitors, averages for insect-exposed treatments were subtracted from the average number of pollen grains counted on a stigma that had no insect visitation. Each treatment was sampled from 20 inflorescences bagged prior to blooming. Bags were removed once flowers opened and stigmas were exposed. Numbers of stigmas collected from each branch for pollen counting were as reported in Table 2.6. Stigmas in this experiment were collected, placed into individual collection tubes, and then put on ice to prevent pollen germination. Once in the laboratory, stigmas were stored in a -20°C freezer until pollen counting could be done. McGillivray's (1987) method for removing pollen from an insect was modified to remove pollen grains from stigmas. Glycerol gelatin was melted in a hot water bath, and three drops of melted gelatin were placed into the bottom of each centrifuge tube (1.5 ml Eppendorf). Tubes were set aside to allow the gelatin to congeal. 20% alcohol was added to the collection tube containing the stigma and then the stigma and alcohol transferred into the centrifuge tube containing the gelatin.

The collection tubes were rinsed with more alcohol and added to the centrifuge tube now containing the stigma. Centrifuge tubes were vortexed for 30 seconds, and then stigmas were removed. Several stigmas were set aside to view under a microscope to ensure efficient removal of pollen grains had been achieved after being vortexed. Centrifuge tubes now containing pollen and alcohol were centrifuged at 13000 rpm for two minutes. Supernatant liquid was decanted and the gelatin with imbedded pollen was removed using a wire with a hooked tip, and placed onto a cover-slip and melted on a hot plate. Cover-slips were used instead of slides because cover slips are much thinner and therefore, pollen grains and the counting grid could be seen simultaneously. When the gelatin was melted the cover slip was removed from the hotplate and the gelatin was allowed to cool slightly. Then another cover slip was placed onto the cooled gelatin and both cover slips containing the gelatin were placed onto the hotplate in order to re-melt the gelatin and create a thin, even layer of gelatin between the two cover slips. Prepared cover-slips were stored in the refrigerator until counted. Cover-slips containing pollen samples were placed onto a hemacytometer plate which had an etched 1 mm^2 grid on its surface and place under a compound microscope for counting (Figure 2.4C). Each cover-slip was divided into sixteen squares and a single 1 mm^2 in the center of each of the sixteen squares was counted and the number of pollen grains was estimated for each sample.

2.3.7. Statistical Analysis

Data collected for yield was subjected to a Proc Mixed analysis using SAS 9.2 (SAS Institute Inc. Cary, NC). Means from open-pollination and insect exclusion in Years I and II were combined. Means were separated using Students *t*-tests. The coefficient of variation was calculated for inflorescence length and number of flowers per inflorescence using SigmaStat 9.1 (Systat Software Inc. San Jose, CA). Fruit set, fruit retention, and fruit quality data were subjected to analysis of variance, and means were separated using Students *t*-tests for Year I and II using SAS 9.2 (SAS Institute Inc. Cary, NC). Data for insect pollen removal were subjected to analysis of variance, and means were separated using Waller-Duncan pairwise comparisons in SAS 9.2 (SAS Institute Inc. Cary, NC).

2.4. Results

Richness and Abundance of Insect Visitors. Thirteen species of insects belonging to four orders (Diptera, Hymenoptera, Lepidoptera, and Coleoptera) were found foraging on macadamia inflorescences (Table 2.2). Among the Diptera were 3 species in Syrphidae (hoverflies), 2 species in Ceratopogonidae (midges), 1 species each in Muscidae, Milichiidae, Calliphoridae, and Chloropidae. Among the Hymenoptera, 2 of the species recorded were *A. mellifera*, and *Xylocopa sonorina* in Apidae. Among the Lepidoptera, 1 species *Tmolus echion* (Larger lantana butterfly), and among the Coleoptera, 1 species *Curinus coeruleus* (Metallic blue lady beetle) was recorded. Only one native insect was seen on the macadamia inflorescences in this orchard, the midge *Forcipomyia hardyi.* During the transect analysis, *A. mellifera* was the most abundant insect visitor, and accounted for 62.7% of all the insect visitors. The next most abundant species visiting macadamia inflorescences was *Ornidia obesa* (Syrphidae), which accounted for 21.2% of all the insect visits. During the focal analysis (Table 2.2), *A. mellifera* and *Conioscinella formosa* (Chloropidae) were equally frequently observed (46.2% of all insects seen). Five insect species recorded during transect insect counts were not observed during the focal insect counts.

Insect forager type (pollen, nectar, or both pollen and nectar), was recorded during focal and transect counts (Table 2.3.). *Apis mellifera*, the syrphids, and *Rhinia apicalis* (Calliphoridae) were the most frequent foragers during the transect analysis. For the focal analysis, *A. mellifera*, and the syrphids were the most frequent foragers. Nectar was preferred to pollen for all of the insects seen with the exception of *A. obliqua. Conioscinella formosa* was difficult to include in this analysis because they were rarely observed foraging for pollen or nectar. Insects with an abundance of less than 1% were not included in Table 2.3.

During focal insect counts, observations were made of insects that had a tendency to foraging on flowers that had the style emerging from the perianth but the stigma and pollen have not emerged. Only open flowers (stigma and pollen accessible) and flowers that would open the next day (stigma and pollen concealed) were included in this study. Both syrphids and honeybees were able to forage on both stages of flowering (Table 2.4).

Impacts of Insect Pollination on Macadamia Fruit Set, Fruit Retention, Nut Quality, and Yield. The coefficients of variation for length of an inflorescence (averaging 15.40 cm ± 0.54) and number of flowers per inflorescence (averaging 187.29 ± 5.38) were small, 0.172% and 0.141%respectively. The low variability observed showed that despite length of inflorescence the number of flowers on the inflorescence was relatively constant, therefore the fruit set and retention was estimated by counting the number of fruits retained per inflorescence.

Open-pollination consistently produced higher fruit set and fruit retained per raceme in both years (Figure 2.5 A, B). For Year 1, OP had three times higher fruit set than IE at 14 days after flowering ($P < 0.001$). At harvest, fruit retention in the OP treatment was 7 times higher per raceme than the IE treatment at harvest ($P < 0.001$). For Year II, OP had 11 times higher fruit set than IE at 14 days after flowering ($P < 0.001$). At harvest, OP had 14 times higher fruit retained than IE ($P < 0.001$). In Year I, fruit abortion stopped at 62 days after flowering and in Year II, 84 days after flowering. This could be verified because there was no change in the number of fruit from the last time abortion was observed until harvest.

In Year I macadamia nuts included in this study were harvested between September 2010 and January 2011, and for Year II harvest began in September 2011 and finished in March 2012. The assessment of treatment effect over both seasons showed significant difference for yields per branch between OP and IE ($P < 0.0001$, Figure 2.6). In terms of quality for Year I, shell width, shell length, and nut in shell weight was significantly higher in IE ($P < 0.05$, Table 2.5). However, in Year II, no difference was observed between OP and IE among all nut quality parameters ($P > 0.05$).

Efficacy of Insect Pollen Removal. The method used to estimate the amount of pollen removed by insects did not allow for differentiation between original pollen and pollen that may have been left behind by a foraging insect; however, since the stigma was not immediately receptive and therefore not sticky, it is likely that very few pollen grains would have been left behind by foraging insects. Newly emerged stigmas are also covered heavily with pollen from the anthers of the same floret, so it is more likely pollen is being removed than being transferred onto stigma. The honeybee and *Ornidia obesa* (Syrphidae) were the predominant flower visitors, and thus were the only insects reported

in Table 2.6. Without insect visitation, an open flower had a mean of 15,298 (±189.9) pollen grains on a stigma. Stigmas exposed to insects all day, or a single visit from a honeybee foraging for pollen removed significantly more pollen compared to the other treatments ($P < 0.0001$). However, a single visit from an insect foraging for nectar (*Ornidia obesa* or the honeybee) was not significantly different compared to stigmas that had no insect visitation ($P > 0.05$).

2.5. Discussion

Richness and Abundance of Insect Visitors. Based on this study, Diptera contributed the highest number of species of flower visitors, but their abundance was low, with the exception of *C. formosa*. Although *C. formosa* was high in abundance during focal insect counts, its role in pollination appeared to be small in this orchard, based on observations. During the first year of this study, some of the bags used to exclude insects were not of a fine enough weave to exclude all insects, and permitted *C. formosa* to visit bagged racemes, which they were observed to do in great numbers, yet the fruit set in these bags yielded one fruit at best or none, similar to what we saw in inflorescences that had all insects excluded. Heard and Exley (1994) found that smaller insects have a lesser contribution to pollination if they rarely visit flowers, and do not make contact with the stigma and the anthers while foraging for nectar. *Conioscinella formosa* (1.5mm) was the observed visiting macadamia nut florets, and was often seen deep in the inflorescence or just resting on the inflorescence, thus meeting the criteria for not being an effective contributor to pollination. Along with size and behavior, vector abundance is an important factor in determining contribution to pollination. *Apis mellifera* was the most dominant species for both transect and focal counts in this orchard. Due to their abundance *A. mellifera* was likely a high contributer to pollination of macadamia in the study orchard.

Although the two methods used to measure insect abundance showed similar trends, some of the insects were differentially detected by the different methods. In this study, the transect analysis detected higher species richness; however, obtaining accurate abundance levels of very small insects using this method may be difficult as was the case with *C. formosa,* the smallest insect observed visiting the flowers, pale yellow in color

and often found deep inside the macadamia inflorescences, possibly allowing them to go unseen during the transect analysis. Although the focal analysis was superior for recording cryptic insects, it missed some of the insects that are scarce in the orchard. While the transect analysis provided information on species richness, and abundance, focal observations elucidated insect foraging behavior on the inflorescences, such as forager flexibility while foraging for nectar. Honeybees and the syrphid *O. obesa* were both capable of foraging on flowers of different flower stages, flowers with only nectar accessible and flowers with both pollen and nectar accessible. Similar results for the abundance of pollen and nectar foragers were recorded during both of the analyses.

The syrphid, *Allograpta obliqua* was the only insect recorded to forage more for pollen than nectar. Some syrphids will forage for nectar as well as pollen. In our study, *A. obliqua* was seen mostly on the stigma of macadamia, and at times appeared to be sucking on the pollen. Interestingly the syrphid flies have specialized mouth parts that are capable of lacerating pollen grains to extract nutrients or ingesting whole pollen grains (Lundgren, 2009). Schneider (1969) and Haslett (1989) showed that pollen is important in normal egg production for several species of syrphids, which could be true for *A. obliqua*. Other Syrphidae such as *O. obesa,* were mostly foraging for nectar and rarely on the stigmas.

There were a high number of recorded instances in which flies spent time not foraging for pollen or nectar while on an inflorescence. During these instances flies were seen to be resting on the macadamia inflorescences. In contrast, the only time honeybees were seen on an inflorescence not foraging for anything was when they were grooming. The difference seen between these insects foraging could be explained by the difference in level of sociality. The honeybee is a eusocial animal, the highest of social ranking. A foraging honeybee is not foraging just for herself, but for the tens of thousands of honeybees in the colony; whereas, the flies forage for individual sustenance and may not have the drive or need to forage as continuously.

Macadamia Fruit Set, Yield and Nut Quality. Based on the results from the fruit set and fruit retention experiment, insect pollination can significantly increase both. Macadamia nut trees are biennial, meaning that the trees will alternate year to year, from producing a light crop to producing a heavy crop and could explain the increased fruit set

observed in the OP treatment for 2011. Different factors, such as, plant management, weather, and nutrients can cause variations in crop production (Huett, 2004; Stephenson and Cull, 1986). Yields were considerably higher in inflorescences pollinated by insects than those for which insects were excluded. It is clear that insects were important for increasing macadamia nut yields. However, in terms of quality, nut size and weight, was not increased with insect pollination.

Trueman and Turnbull (1994a) showed an increase in nut mass with hand cross-pollination within 'Kau 344' and 'Keaau 660'. It could be that the amount of pollen transferred during hand-pollination from one tree to another tree exceeds the amount an insect will typically transfer while foraging among trees. There was a significant difference for the first year in some quality measurements for IE. The larger fruits obtained in the IE treatment could be due to the fact that inflorescences in the IE treatment never produced more than two nuts on one inflorescence, where OP treatments often exceeded two nuts per raceme and could have up to nine nuts per raceme. Having one or two nuts on an inflorescence would mean that nutrients for that inflorescence would not have to be divided to the degree that had otherwise been seen in inflorescences in the OP treatment.

Efficacy of Insect Pollen Removal. Honeybees foraging for pollen removed the most pollen per visit compared to other insect forager types. Although they removed large amounts of pollen, the proportion of honeybees foraging for pollen was low. The majority of their visits were for nectar. Insects foraging for nectar removed similar amounts of pollen compared to stigmas that received no insect visitation. The low amount of pollen removed by insects foraging for nectar indicates that multiple insect visitations from nectar foragers are needed to remove most of the pollen from the stigma. The high percentage of nectar foragers combined with their low pollen removal rate could be advantageous to the plant. For nectar foragers to have significant pollen removal from a stigma many visits are required and therefore the chances for cross-pollination to occur would be increased.

Exely et al. (1988) concluded that insects foraging mostly for pollen among macadamia inflorescences can contribute significantly to macadamia pollination due to their frequent contact with the stigma. The only insect in our study recorded to forage for

mostly pollen was the syrphid *Allograpta obliqua.* Although syrphids have the potential to be a major contributor to pollination in certain crops (Jauker et al., 2012; Schittenhelm et al., 1997), in this orchard they lacked some of the characteristics that honeybees exhibited. Syrphids were seen in a lower abundance, and when foraging for neither pollen nor nectar, will often avoid touching stigmas. Jauker et al. (2012) reported that for syrphid pollination to result in similar fruit set and yields mediated by bees, they would need to be present in high densities (five times that of bees). In large macadamia orchards in Hawaii this might be difficult to achieve.

In conclusion, the honeybee was the most abundant flower visiting insect and appeared to contribute extensively to the movement of pollen for macadamia nuts. Overall, the results indicate that it is important to have insect visitation to increase macadamia fruit set, and fruit retention. Thus, the main implication of this study is that macadamia farmers would benefit from maintaining honeybee hives in macadamia orchards. Although increasing honeybees within an orchard would be the easiest way to improve a low nut yield due to insufficient pollination in Hawaii, the contribution to pollination that could be gained from increasing syrphids in an orchard could be a beneficial addition to macadamia pollination.

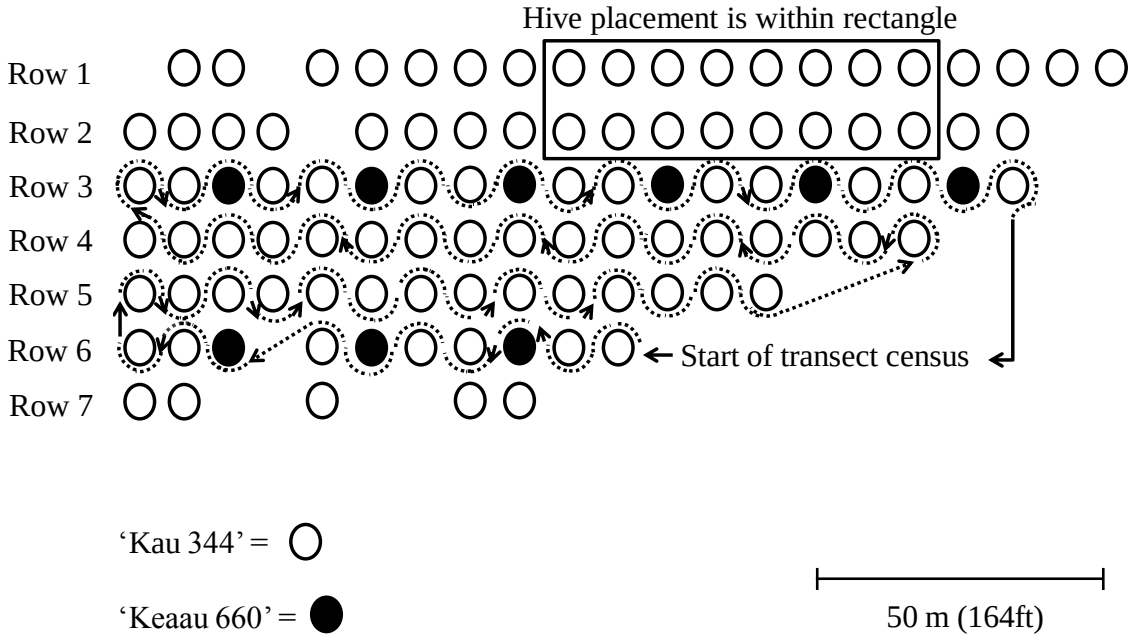

Figure 2.1. Map of orchard at the University of Hawaii Waimanalo Research Station showing transect route.

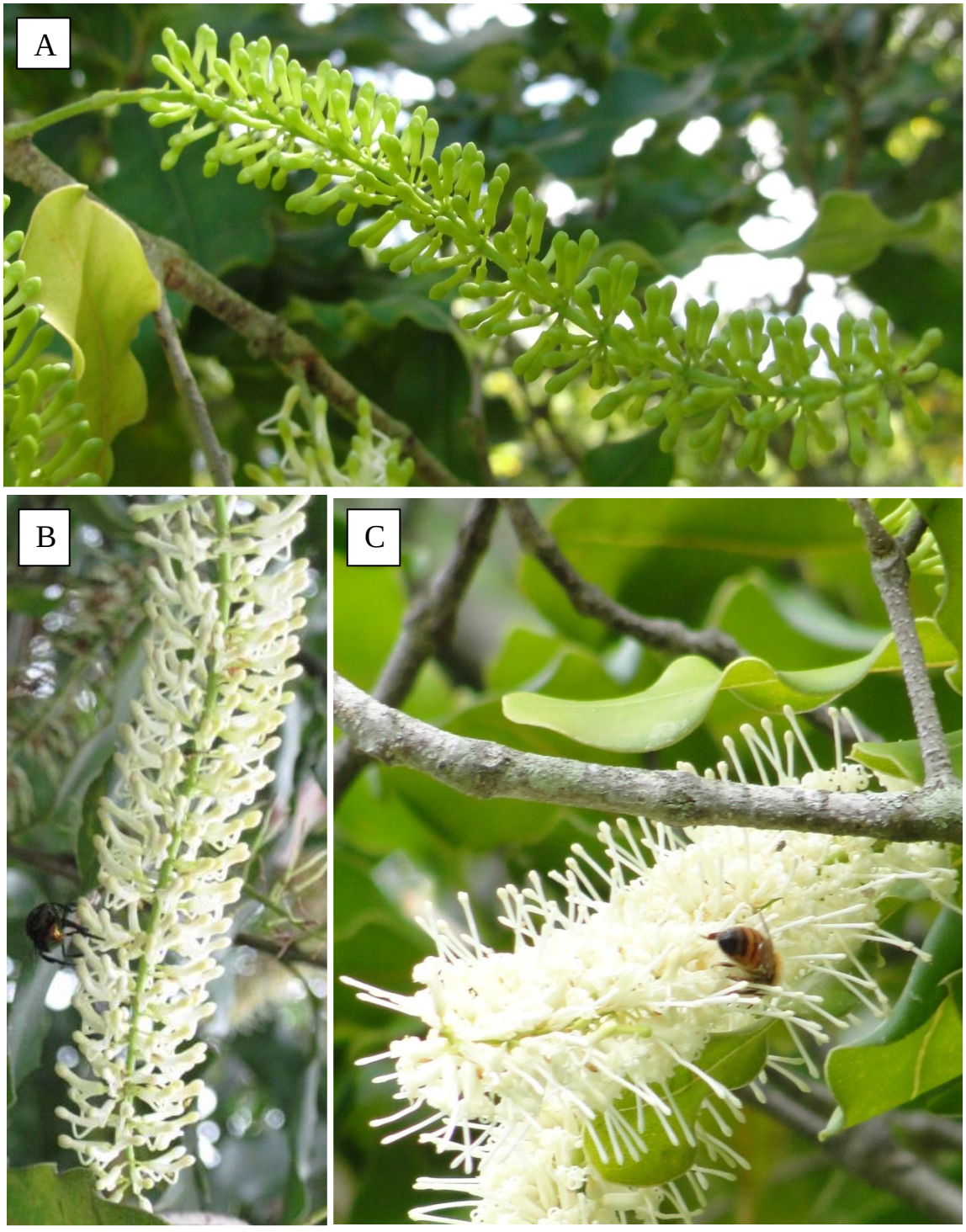

Figure 2.2. A) Immature macadamia inflorescence, B) macadamia inflorescence one day before opening with a syrphid, *Ornidia obesa*, and C) macadamia inflorescence that has flowers completely open with a honeybee, *Apis mellifera*.

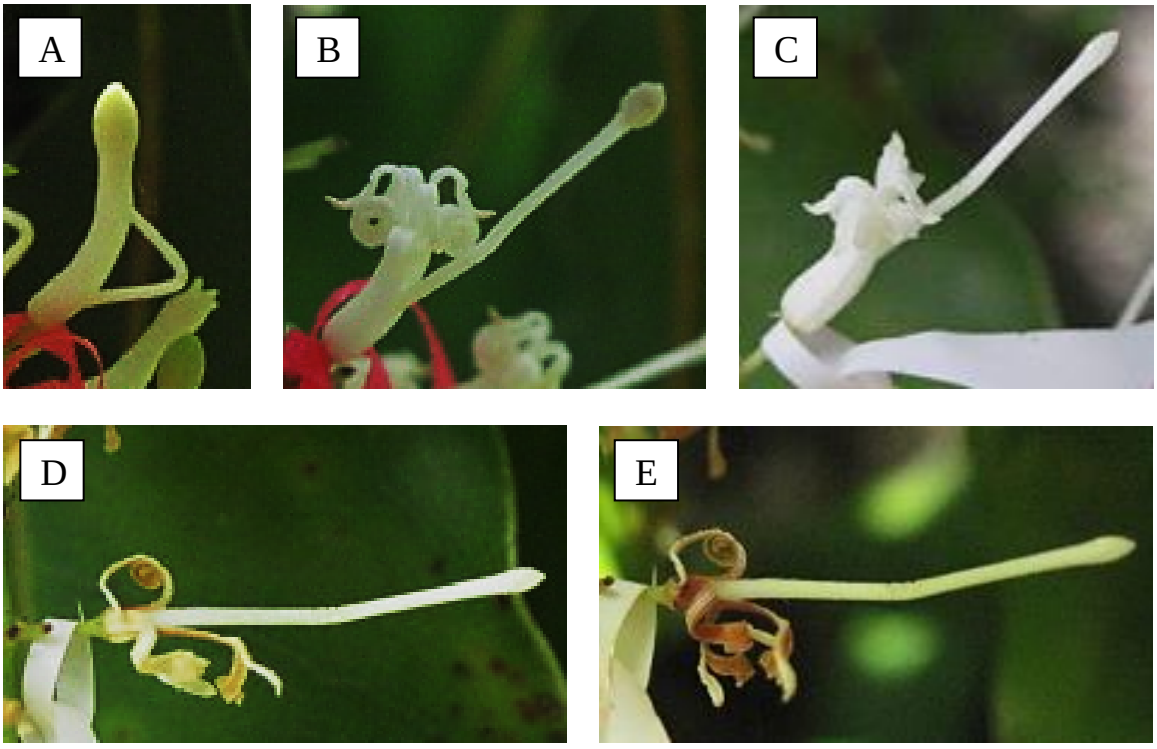

Figure 2.3. Progression of a macadamia flower: A) macadamia flower one day before opening with its pistil beginning to emerge from perianth and creating a loop, B) macadamia flower that has just opened, note pollen on the stigma, C) two days old, note stigma is free of pollen, D) three days old, and E) four days old, petaloid sepals have turned brown and will soon drop off. Stigma is receptive around 2-3 days.

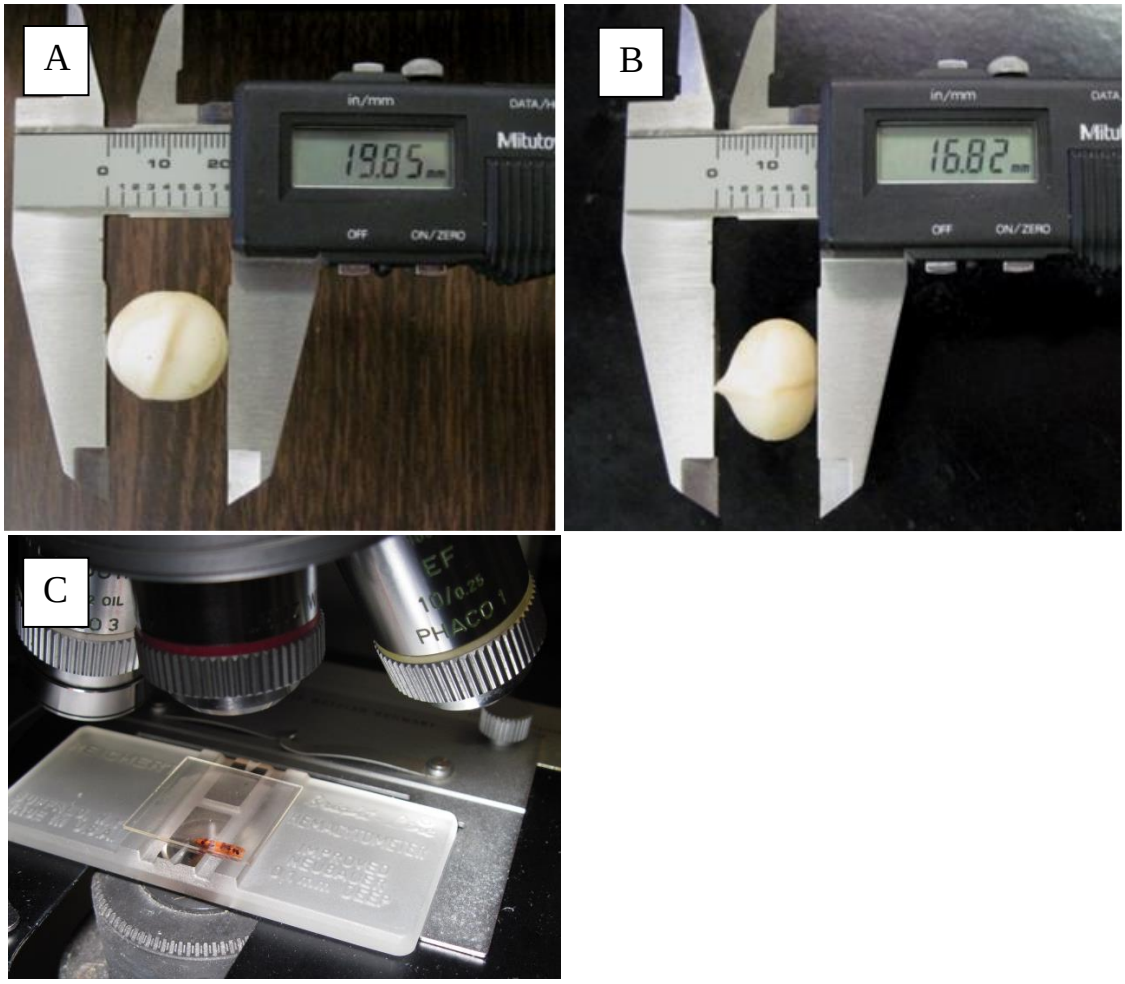

Figure 2.4. A) width of the nut (widest width), B) length of the nut, and C) counting pollen grains with compound microscope and hemacytometer plate.

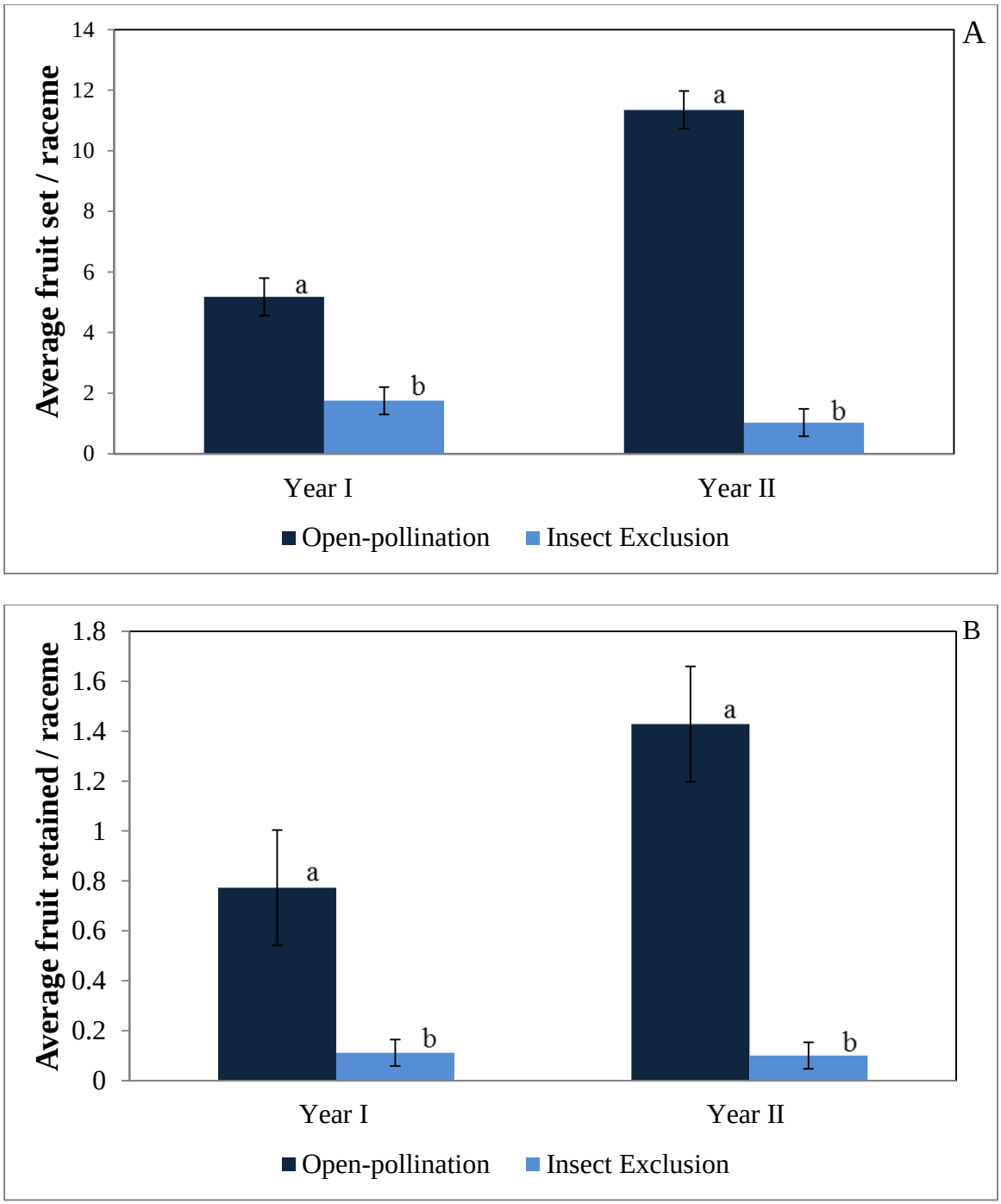

Figure 2.5. A) Initial fruit set per raceme at 14 days after flowering, and B) fruit retention at 2 months (2010, n = 10) and 3 months (2011, n = 40) after flowering. Error bars shown are standard errors. Bars with different letters (within a year) were significantly different ($P < 0.001$) based on Students *t*-tests.

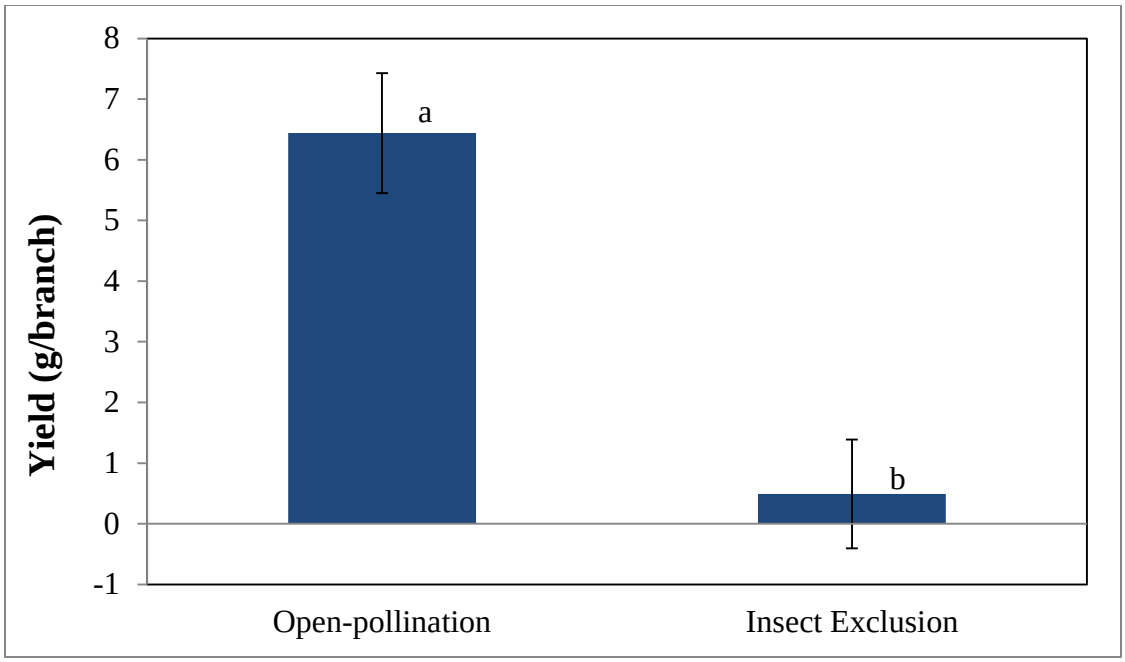

Figure 2.6. Macadamia yield of open-pollination and insect exclusion for Year I and II combined. Error bars are standard errors. Bars with different letters were significantly different ($P < 0.0001$) based on Students *t*-tests.

Table 2.1. Examples of transect and focal insect count recording sheet used for field data collecting

Transect Insect Counts						
Waimanalo 3/16/10 7am-8am	Inflorescence Stage	*Apis mellifera*	*Ornidia obesa*	*Allograpta obliqua*	*Conioscinella formosa*	Nectar/ Pollen
7:00	All open	1				N
	¾ open			1		P
	½ old	1				N
7:05	All open	2				N
	1/3 open	1				N
	¼ open		1			N
	All closed	1				N
7:10	All open	1				P
	1/2 open	1				N
	1/3 open				3	Neither
7:11	All open	1				N

Focal Insect Counts						
	Inflorescence Stage & Sun Exposure	Nectar or Pollen	*Apis mellifera*	*Ornidia obesa*	*Conioscinella formosa*	Observation Notes
9:00	2/3 open	N	1			Open flowers only
↓	1/3 Pistil looping (PL)	N	1			Open flowers only
↓	Part sun shade	N	1			On both open and PL flowers
↓		N		1		On PL flowers only
↓			1		5	Stayed on petals and inside corolla
		N	1			On PL flowers only
9:20		N	1			On both

Table 2.2. Abundance of insect visitors observed visiting macadamia inflorescences during transect and focal counts at the University of Hawaii Waimanalo Research Station, Oahu, Hawaii

	Transect		Focal	
Insect Taxa	Total sightings	Abundance (%)	Total sightings	Abundance (%)
Apis mellifera (Apidae)	745	62.7	154	46.2
Xylocopa sonorina (Apidae)	7	< 1	-	-
Ornidia obesa (Syrphidae)	252	21.2	12	3.6
Eristalis arvorum (Syrphidae)	56	4.7	7	2.1
Allograpta obliqua (Syrphidae)	17	1.4	2	< 1
Conioscinella formosa (Chloropidae)	81	6.8	154	46.2
Rhinia apicalis (Calliphoridae)	20	1.7	1	< 1
Musca domestica (Muscidae)	2	< 1	-	-
Forcipomyia hardyi (Ceratopogonidae)	1	< 1	1	< 1
Desmometopa sp. (Milichiidae)	1	< 1	1	< 1
Atrichopogon jacobsoni (Ceratopogonidae)	1	< 1	-	-
Curinus coeruleus (Coccinellidae)	1	< 1	1	< 1
Tmolus echion (Lycaenidae)	4	< 1	-	-

Table 2.3. Incidence of each forager type observed during inflorescence observations during abundance analyses from 6:00 am to 5:00 pm over a period of 3 days for Year I and 5 days for Year II (n = number of insects). Includes all stages of flowers that were attractive to insects.

	Percent forager type		
Insect visitor/Forage Type	Year I Transect Analysis	Year II Transect Analysis	Year II Focal Analysis
Apis mellifera	(n = 265)	(n = 742)	(n = 151)
Nectar	97.0	95.8	93.8
Pollen	3.0	4.2	6.2
Ornidia obesa		(n = 155)	(n = 10)
Nectar	-	100.0	100.0
Pollen	-	0.0	0.0
Eristalis arvorum		(n = 37)	
Nectar	-	89.2	-
Pollen	-	10.8	-
Allograpta obliqua		(n = 8)	
Nectar	-	25.0	-
Pollen	-	75.0	-
Rhinia apicalis		(n = 14)	
Nectar	-	85.7	-
Pollen	-	14.3	-

Table 2.4. Foraging of individual honeybees and syrphids (*O. obesa*) during the focal counts on macadamia nut inflorescences that had flowers one day before opening with pistils emerging from perianth, and flowers that were open with pollen and nectar available to insects

	% Foraging on flowers with pistil emerging. No stigma contact.	% Foraging on open flowers.	% Foraging on both flower types: open and pistil emerging.
Honeybee (n = 77)	13.0	51.9	35.1
Syrphid *O. obesa* (n = 13)	53.8	15.4	30.8

Table 2.5. Mean (±SEM) for fruit and kernel parameters (mm) and mass (g) for macadamia pollination experiments at University of Hawaii Waimanalo Research Station, HI

	Year I Treatments	
	Open-Pollination	Insect Exclusion
Mean shell diameter	23.04B (±0.36)	24.87A (±0.33)
Mean shell length	21.91B (±0.33)	24.12A (±0.21)
Mean nut diameter	18.56A (±0.35)	19.93A (±0.29)
Mean nut length	15.30A (±0.37)	15.84A (±(0.90)
Mean nut in shell weight	6.31B (±0.24)	7.80A (±0.18)
Mean kernel weight	2.19A (±0.12)	2.64A (0.11)
	Year II Treatments	
	Open-Pollination	Insect exclusion
Mean shell diameter	25.32A (±0.19)	24.96A (±1.28)
Mean shell length	23.84A (±0.17)	24.37A (±0.89)
Mean nut diameter	20.55A (±0.18)	20.56A (±1.20)
Mean nut length	17.56A (±0.16)	16.82A (±1.26)
Mean nut in shell weight	8.68A (±0.18)	9.08A (±1.19)
Mean kernel weight	3.08A (±0.07)	3.00A (±0.51)
Mean dry kernel weight	2.67A (±0.06)	2.52A (±0.43)

[a] Treatment means with different letters in the same row were significantly different ($P < 0.05$) based on students *t*-tests.

Table 2.6. Pollen removal rate of insects on insect exposed macadamia flowers.

Insect visit time	Sample #	Mean pollen grains / stigma (±SEM)	% pollen grains removed by insects
All day	27	795.5A[a] (±189.9)	94.8
Honeybee one visit (Pollen)	7	2938.6A (±1314.4)	80.8
Honeybee one visit (Nectar)	23	13901.9B (±1447.0)	9.1
O. obesa one visit (Nectar)	5	14514.0B (±2781.5)	5.1
0 visit	22	15298.3B (±1379.2)	0.0

[a] Treatment means with different letters in the same column were significantly different ($P < 0.0001$) based on Waller-Duncan tests for pairwise comparisons. To estimate the amount of pollen removed by the insect visitors, averages for insect-exposed treatments were subtracted from 15298.3.

2.6. References

Abe, T., K. Wada, Y. Kato, S. Makino, and I. Okochi. 2011. Alien pollinator promotes invasive mutualism in an insular pollination system. Biol Invasions. 13: 957-967.

Coetzee J. H., J. H. Giliomee. 1985. Insects in association with the inflorescence of *Protea repens* (L.) (Proteaceae) and their role in pollination. Journal of the Entomological Society of South Africa 48: 303–314.

Drake, E. F. 1971. Life cycle and laboratory diet for *Atrichopogon jacobsoni* (de Meijere) (Diptera: Ceratopogonidae). Proceedings of the Hawaiian Entomological Society. 21: 63-66.

Haslett, J. R. 1989. Adult feeding by holometabolous insects: Pollen and nectar as complementary nutrient sources for *Rhingia campestris* (Diptera: Syrphidae). Oecologia. 81: 361-363.

Hattingh, V. and J. H. Giliomee. 1989. Pollination of certain *Leucadendron* species (Proteaceae). South African Journal of Botany. 55: 387-393.

Heard, T. A., and E. M. Exley. 1994. Diversity, abundance, and distribution of insect visitors to macadamia flowers . Environmental Entomology. 23: 91-100.

Huett, D. O. 2004. Macadamia physiology reviw: a canopy light response study and literature review. Australian Journal of Agricultural Research. 55: 609-624.

Jauker, F., B. Bondarenko, H.C. Becker, and I. Steffan-Dewenter. 2012. Pollination efficiency of wild bees and hoverflies provided to oilseed. Agricultural and Forest Entomology. 14: 81-87.

Kaufmann, T. 1974. Behavioral biology of a cocoa pollinator, *Forcipomyia inornatipennis* (Diptera: Ceratopogonidae) in Ghana. Journal of the Kansas Entomological Society. 47: 541-548.

Lundgren, J. G. 2009. *Relationships of Natural Enemies and Non-Prey Foods, Progress in Biological Control &.* Springer Science+Business Media B. V.

McGillivray, D. B. 1987. A centrifuging method for the removal of insect pollen loads. Journal of the Entomological Society of Southern Africa. 50: 521-523.

McGregor, S. E. 1976. Insect pollination of cultivated crop plants*:* Small fruits and brambles. http://www.culturaapicola.com.ar/apuntes/libros/Polinizacion /index.html.

(NASS) National Agricultural Statistics Services. 2012. Hawaii Agriculture 2011. USDA, Hawaii Field Office. Honolulu, HI. http://www.nass.usda.gov/Statistics _by_State/Hawaii/Publications/Miscellaneous/hiag.pdf.

Roubik, D. W. 1995. Pollination of cultivated plants in the tropics, FAO Agricultural Services Bulletin 118. Food and Agriculture Organization (FAO). Rome, Italy.

Roubik, D. W. 2002. Feral african bees augment neotropical coffee yeild, pp. 255-266. *In* P. Kevan and V. L. Imperatriz Fonseca (eds.), Pollinating Bees - The Conservation Link Between Agriculture and Nature. Ministry of Environment, Brasil.

Rourke, J., and W. Delbert. 1977. Convergent floral evolution in South Africa and Australian Proteaceae and its possible bearing on pollination by nonflying mammals. Annals of the Missouri Botanical Garden Press. 64: 1-17.

Schittenhelm, S., T. Gladis, and V. R. Rao. 1997. Efficiency of various insects in germplasm regeneration of carrot, onion and turnip. Plant Breeding. 116: 369-375.

Schneider, F. 1969. Bionomics and physiology of aphidophagous syrphidae. Annual Review of Entomology. 14: 103-124.

Stephenson, R. A. and B. W. Cull. 1986. Effects of site, climate, cultivar, flushing and soil and leaf nutrients on yields of macadamia in South east Queensland. Scientia Horticulturae. 30:227-235.

Trueman S. J., and C. G. N. Turnbull. 1994a. Effects of cross pollination and flower removal on fruit set in macadamia. Annals of Botany 73: 23-32.

Trueman, S. J., & Turnbull, C. G. 1994. Fruit set, abscission and dry matter accumulation on girdled branches of macadamia. Annals of Botany. 74: 667-674.

(USDA) United States Department of Agriculture. 2012. State Fact Sheets: Hawaii. USDA, Economic Research Service. http://www.ers.usda.gov/statefacts/HI.HTM #TCEC.

Wallace, H. M., V. Vithanage, and E. M. Exley. 1996. The effects of supplementary pollination on nut set of macadamia (Proteaceae). Annals of Botany. 78 (6): 765-773.

Willmer, P. (2011). Pollination and Floral Ecology. Princeton University Press, Princeton, NJ.

Wright, M. G., D. Visser, J. H. Coetzee, and J.H. Giliomee. 1991. Insect and bird pollination of Protea species in the western Cape: Further data. South African Journal of Science. 87 (5): 214-215.

CHAPTER 3
CONTRIBUTION OF INSECT POLLINATION TO *COFFEA ARABICA* IN HAWAII

3.1. Abstract

Apis mellifera (the honeybee) is an important pollinator in many agricultural crop systems in Hawaii; however, its contribution to pollination, along with other insect visitors, has not been determined for coffee in Hawaii. To determine the extent of insect pollination in coffee and its effect on fruit production, the following were investigated in a local coffee plantation: 1) species richness and abundance of insects visiting flowers; 2) effects of insect pollination on fruit set, fruit retention, bean size, and mass; and 3) pollen transfer efficacy of insects. Results indicate that flies (Order Diptera) were the more diverse group at the study site (8 species) compared to 2 species of bees (Order Hymenoptera). In spite of the greater species richness in Diptera, *A. mellifera* was the most abundant species vising the coffee flowers (86.4% in abundance, with an average of 35 honeybees seen per 15 minutes compared to 3 flies per 15 minutes). Insect pollination resulted in higher fruit set, fruit retention, and yield compared to branches from which insect visitation was excluded. The results of pollen transfer showed that a single visit from a honeybee doubled the amount of pollen that was deposited on a stigma, and stigmas exposed to *ad libitum* insect pollination received about twice as many pollen grains as those exposed to a single honeybee visit. Honeybees were the greatest contributor to pollination in the coffee plantation.

3.2. Introduction

Coffee (*Coffea arabica*, Rubiaceae) is an important crop for Hawaii, producing $31.5 mil farm gate value in 2011 (NASS, 2012). Honeybees (*Apis mellifera*) play an important role in pollination for a wide range of agricultural crops (Roubik, 1995). Pollination by honeybees is often taken for granted, as feral colonies are able to provide effective pollination. In many cases, their contribution has never demanded research

attention. However, dramatic losses of feral and managed honeybee colonies have relatively recently occurred in Hawaii, largely attributable to the invasion of *Varroa destructor* (varroa mite) in 2007 and *Aethina tumida* (small hive beetle) in 2010 (Connor, 2011). A decrease in pollinators can cause farmers to experience great reductions in crop yield. Economic losses from the loss of honeybee colonies are especially known to be damaging for crops requiring cross-pollination (Sass, 2011). However, the impact of reduced honeybee abundance on self-pollinated crops, such as coffee, is still debatable (Philpott et al., 2006; Ricketts, 2004; Klein, 2009; Klein et al., 2003). This chapter focuses on evaluating the contribution of insect pollination on coffee in Hawaii. Specific objectives of this study were to examine: 1) species richness and abundance of potential pollinators visiting coffee flowers, 2) the impacts of insect pollination on coffee fruit quantity and quality, and 3) the efficacy of insects to transfer pollen to the stigma while foraging on a coffee flower.

3.3. Materials and Methods

3.3.1. Study Site

This study was conducted in a commercial coffee plantation in Waiahole (21˚48' N, 157˚87' W) on the island of Oahu, Hawaii, between March and November of 2010 (Year I), and between March 2011 and January 2012 (Year II). The study plot comprised 67 coffee trees planted in six rows, encompassing approximately 60×20 m^2 in partial sun (Figure 3.1). Four managed beehives were located on the west side of the plantation, approximately 6 m from the western edge of the plantation.

3.3.2. Monitoring Insect Foragers for Species Richness and Abundance

Monitoring of insect foragers was conducted between 8:00 am and 4:00 pm when flowers were blooming both years. Species richness was assessed as described in Chapter 2. Insects were identified to species level, and voucher specimens were deposited in the University of Hawaii Insect Museum. In addition to species richness, abundance of each species was also recorded in Year II (2011) between April 9 and 10, and between May 6 and 7, corresponding to the largest flowering peak (about 8.0 flowers per branch) during

the season. Smaller flowering peaks consisted of about 2.3 flowers per branch, and had very few insect visitations. Most of these larger peaks occurred during cloudy and occasionally misty days. Coffee flowers usually only lasted for two days. One-day old flowers were entirely white (Figure 3.2B). Anthers from day-one flowering would turn brown by day two (Figure 3.2C), but new sets of flowers would bloom on day two, providing a fresh source of pollen. By day three and four, entire flowers wilted (Figure 3.2D) and insect foraging declined precipitously. Therefore, no counts were made three days after plants began flowering.

Insects were monitored using a transect count method following a route through the study plot as illustrated in Figure 3.1, similar to that described in Chapter 2. All trees in the plantation were included in the study except those that were too young and not flowering, indicated by the black symbols (Figure 3.1). The route was walked at a constant pace (average of 80 seconds per plant), monitoring was limited to flowers that were about 1.4 meters above ground. About 225 flowers per plant were observed within the 80 seconds. An average of four insects was observed foraging on flowers during this time frame.

3.3.3. Fruit Set and Fruit Retention

To assess impact of insect visitation on coffee bean production, coffee branches with flower buds (Figure 3.2A) averaging 1.4 m above ground were used. These branches were either: 1) bagged and wire-tied with a fine mesh cloth (measuring about 71.1 cm long × 48.3 cm wide) covering about 50.3 cm of the branch, the "insect exclusion" (IE) treatment; or 2) tagged with flagging tape but not bagged during the flower receptive stage, the "open-pollination" (OP) treatment. An additional treatment was done in Year II to assess self-pollination by geitonogamy, and constituted the "hand-pollination" (HP) treatment. Geitonogamy is when a flower is pollinated by pollen from another flower from the same plant (Roubik, 1995). This is a type of pollination caused by the movement of pollen by insects foraging on flowers within a single plant and likely to occur within plants that produce a large number of flowers per plant (Takashi and Gaku, 2003; De Jong et al., 1992). The coffee plant will produce a large amount of flowers at one time and is therefore likely to be subjected to this type of pollination. This treatment

would allow for another comparison for the OP treatment, reinforcing the benefits that insect pollination can have on fruit production or quality. The hand-pollination treatment included restricting insect access to flowers, similarly to the insect-exclusion protocol, but when the flowers opened the bag was removed and the flowers were pollinated using anthers from the same plant. The method used for hand-pollination was done similar to the technique done by Ricketts et al. (2004). The pollen source was known to be free of any pollen contamination by nearby plants, and the anther was used to carefully touch individual stigmas. Once hand-pollination was done, the flower stem was marked with a felt-tip marker and the branch was re-bagged. All flowers that bloomed within the HP treatment were treated in this manner. Fruit set and fruit retention on each experimental branch was recorded throughout the development of the coffee bean from fertilization to harvest (5 months for 2010 and 6.5 months for 2011). Each treatment was allocated to each tree used in the study. To avoid differential effects due to manipulation and bagging on initial fruit development, OP inflorescences were also bagged once flowers were no longer attractive to insects in Year II. Table 3.3 gives a summary regarding what was done to each treatment for both years and the sample size (including branches and flowers) for each treatment. Initially 10 branches per treatment were used, but during the study, four branches in the IE treatment and two branches in the OP treatment were lost due to branch dieback. Initially 20 branches per treatment were used, but during the study one branch in the IE treatment, one branch in the OP treatment, and three branches in HP were lost due to storms and each treatment lost one branch due to dieback.

For Year I, fruit retention was recorded each week until records determined that fruits were no longer being lost. For Year II, fruit retention was recorded every three weeks until records determined fruits were no longer being lost. Branches were considered to not lose any more fruits when three consecutive fruit recordings showed no fruits were being lost. Fruits were harvested weekly when they turned completely red and/or slightly soft when squeezed. All fruits harvested were accumulated as total yield.

3.3.4. Bean Quality

Bean quality was assessed by recording length, and dry weight of individual beans harvested. Beans were dried at 38°C for three days in an incubator. To determine

the length of time needed for the beans to dry at this temperature, beans were weighed also at day four. At day four bean weights remained consistent, therefore all beans were dried for three days and then weighed. The length of fruits and beans were measured using digital calipers to the nearest 0.01 mm. These measurements included length of 1) the entire fruit, including the pulp layer, and 2) individual beans, with the pulp removed. Mass of the whole fruits and beans was measured using a digital scale to the nearest 0.01 g. Beans were dried then weighed for final dry-weight values. Another indication of poor pollination is the development of peaberries. Peaberries occur when only one of the two ovules is fertilized, which results in only one bean per fruit to be formed instead of two. Therefore, peaberries were counted, measured and weight along with the normal beans.

3.3.5. Efficacy of Insect Pollen Transfer

The number of pollen grains deposited on the stigmas of flowers was counted after 1) one insect visit, 2) a whole day exposed to insect visitation (6:00am – 3:00pm), or 3) without insect visitation. This assessment is based on the assumption that any increase observed in pollen deposition on the OP flowers was the result of insect activity. Stigmas were collected from 10 coffee branches. These branches were bagged prior to blooming to ensure stigmas had no prior insect visitation. The number of stigmas collected from each treatment for quantifying pollen loads are shown in Table 3.7. Each stigma was collected into a vial and stored on ice to prevent pollen germination. Once transferred into the laboratory, stigmas were stored in a -20°C freezer until processed. The procedure for pollen removal from the stigma was described in Chapter 2 for macadamia nut pollen counts.

3.3.6. Statistical Analysis

Data collected for yield was subjected to a Proc Mixed analysis using SAS 9.2 (SAS Institute Inc. Cary, NC). Means from open-pollination and insect exclusion in Years I and II were combined. Means were separated using Students *t*-tests ($P < 0.01$). Due to the lack of hand-pollination in Year I open-pollination and hand-pollination were compared only in Year II using the method described above. Flowers per node and branch, fruit set, fruit retention, and fruit quality data were subjected to On-way analysis

of variance using Students *t*-tests in Year I, and Waller-Duncan pair-wise comparisons in Year II using SAS 9.2 (SAS Institute Inc. Cary, NC). Data for pollen counts from stigmas were subjected to analysis of variance, log transformation was done to achieve normal distribution, and means were separated using Waller-Duncan pair-wise comparisons in SAS 9.2 (SAS Institute Inc. Cary, NC).

3.4. Results

Richness and Abundance of Insect Visitors. Fourteen species of insects belonging to three orders (Diptera, Hymenoptera, and Lepidoptera) were observed foraging on coffee flowers (Table 3.1). Among the Diptera were six Syrphidae species (hoverflies), 1 Calliphoridae species, 1 Drosophilidae species, and 1 Muscidae. Two of the Syrphidae, *Syritta oceanica* and *S. orientalis*, are difficult to distinguish without a microscope, and consequently they were not identified to species level in field observations, but rather were grouped together in Table 3.1 as *Syritta* spp. Among the Hymenoptera visitors, of the species recorded belong to the Family Apidae: *A. mellifera* and *Xylocopa sonorina*, the other two hymenopterans were ants (Formicidae), specifically *Technomyrmex difficilis* and *Pheidole* sp. The two Lepidoptera species recorded belonged to different families, *Macroglossum* sp. (a hummingbird hawk moth, Sphingidae) and *Papilio xuthus* (the chinese yellow swallowtail, Papilionidae). During the transect analysis, *A. mellifera* was the most abundant insect visitor, (86.3 % of all the insect visitors observed). The next two most abundant species visiting coffee flowers was *Allograpta obliqua* in the Syrphidae family (5.6 % of all insect visits observed) and *Pheidole* sp. in the Formicidae family (4.6% of all insect visits observed). The other insects observed had abundances of less than 1%.

During the insect abundance analysis, insects were categorized on whether they foraged for nectar or pollen. Thus, insect forager type (pollen, nectar, or both pollen and nectar) was recorded (Table 3.2.). *Apis mellifera, A. obliqua* and *Pheidole* sp. were the most frequent foragers. Pollen was preferred over nectar by *Apis mellifera* and *A. obliqua*, but the reverse was true for *Pheidole* sp. Insects with an abundance of less than 1% of observations were not included in Table 3.2.

Impacts of Insect Pollination on Coffee Fruit Set, Fruit Retention, Yield and Bean Quality. In general, the number of flowers produced was higher in Year I (mean = 10.8 flowers per node overall; OP mean = 12.2; IE mean = 9.5; $P < 0.001$) than Year II (4.5 flowers per node, no significant differences between treatments) (Table 3.3). The average number of flowers per branch was not significantly different between the treatments within Year I or Year II ($P > 0.05$, Table 3.3). In Year I, fruit abortion stopped at 70 and 105 days after flowering for OP and IE, respectively. For Year II, fruit abortion stopped at 103 days after flowering for all three treatments.

Open-pollination consistently produced higher fruit set per branch than IE in both Years I and II (Figure 3.3A). In Year I, OP had 19% more fruit set per branch than IE ($P < 0.01$) at 21 days after flowering. At harvest, OP had 5% more fruits retained per branch than IE at harvest and was not significantly different ($P > 0.05$, Figure 3.3B). In Year II, both OP and HP had more fruit set at 21days after flowering and higher fruit retention at harvest than IE ($P < 0.001$, Figure 3.3B). Fruit set was 16% more in OP and 21% more in HP than IE at 21 days after flowering (Figure 3.3A). Fruit retention was significantly higher in HP compared to OP ($P < 0.0001$), and had 40% more fruit retained at harvest compared to IE (Figure 3.3B). Fruit retention was significantly higher in OP compared to IE ($P < 0.0001$), and had 27% more fruit retained at harvest compared to IE (Figure 3.3B).

The assessment of treatment effect over both seasons showed significant difference for yields per branch between OP and IE ($P < 0.01$, Figure 3.4). In Year II, yield per branch was not significantly higher in OP and HP ($P > 0.05$, Figure 3.4).

In terms of fruit quality, fruit and bean length, and mass were significantly lower in the IE than OP in Year I ($P < 0.0001$, Table 3.4). However, in Year II, no difference was observed between HP and IE among all fruit quality parameters ($P > 0.05$). On the other hand, OP had longer bean length, and higher dry bean mass than IE ($P < 0.01$).

The amount of peaberries per branch for both years was highest in the IE treatments. However, for Year I there was no significant difference ($P > 0.05$). For Year II, OP and HP were lowest in peaberries per branch compared to IE ($P < 0.01$). There was no significant difference in the number of peaberries between OP and HP ($P > 0.05$) (Table 3.5). There was also no significant difference between the mass of peaberries

compared to normal beans within the treatments, except in the OP treatment in Year I ($P < 0.05$) (Table 3.6).

Efficacy of Insect Pollen Transfer. The honeybee was the predominant (86%) flower visitor by a large margin, and as such data on efficacy of pollen transfer will be exclusively derived from honeybee observations Table 3.7. Without insect visits, an open flower had an average of 299 pollen grains per stigma. However, a single visit from a honeybee foraging for pollen or nectar could also transfer twice the amount of pollen compared to a stigma that received no insect pollination (Table 3.7). There was no significant difference between the amounts of pollen grains transferred by one honeybee visit compared to stigmas that were exposed all day ($P < 0.05$, Table 3.7).

3.5. Discussion

Species Richness and Abundance of Insect Visitors. Based on this study, Diptera had the highest species richness but lowest abundance in this coffee plantation. Insect abundance plays a key role in effective pollination. A rare flower visitor would likely contribute little to pollination (Roubik, 2002). *Apis mellifera* was the dominant species in this coffee plantation. This is consistent with other findings, that *Apis* spp. are generally the most abundant pollinators, comprising more than half of all insects visiting coffee (Raw and Free, 1977; Roubik, 2002; Vergara and Badano, 2009). Sahil and Conner (2006) showed that pollinator abundance and effectiveness are important factors to determine importance of a pollinator. Due to their abundance and efficiency in pollen transfer, *Apis mellifera* almost certainly contributed the most to pollination of the coffee flowers in the study plantation.

Apis mellifera and *A. obliqua* (the most abundant syrphid and second most abundant flower visitor observed) were the most frequent visitors of coffee flowers. *Apis mellifera* was usually observed collecting pollen (congruent with other studies by Roubik, 2002 and Raw and Free, 1977), whereas *A. obliqua* was usually observed eating pollen. Syrphids are known to consume pollen for nutrients to aid in reproduction (Schneider, 1969; Haslett, 1989). Syrphids have previously been recorded to eat coffee pollen (Ngo et al., 2011). In spite of their taxonomic differences both of these insect visitors are foraging

for pollen to increase their reproductive success. In this study, ants (*Pheidole* sp.) were mostly observed foraging for nectar deep in the coffee flower but not attending the anthers. Philpott et al. (2006) reported that ants helped with coffee pollination; however, whether they contribute directly (transferring pollen to stigma) or indirectly (reducing floral predators or increasing pollinator flower visitation by attacking pollinator) was not determined. Although the current study was not designed to examine this issue specifically, a direct contribution of ants to pollination was not evident during the transect counts as the frequency at which they were seen among the anthers or stigmas was very low. This behavior is not unusual for ants as ants are known to be non-facilitators of pollination by robbing nectar (Ballantyne and Willmer, 2012; Galen, 1999; Ghazoul, 2001). The remaining insects recorded during the transect counts were seen at very low abundances (less than 1%), and may have been present by chance, especially those recorded only once during the transect counts. However, during the transect counts a species of *Macroglossum* (a diurnal moth) was seen throughout the day foraging for nectar. Martins (2008) also reported a *Macroglossum* sp. foraging on coffee flowers and with further study found that while these moths foraged for nectar, coffee pollen was transferred to their body and thus they may facilitate in pollination.

Coffee Fruit Set, Retention, Yield and Bean Quality. Based on the results insect visitation significantly increased the coffee fruit set and fruit retention compared to insect exclusion. In terms of fruit set, open-pollination and hand-pollination performed similarly, and both treatments produced significantly more fruit set than branches excluded from insect visitation. However, the hand-pollination treatment, which ensured pollination to occur within the plant (geitonogamy), resulted in higher fruit retention than open-pollination (which may have included outcrossing by pollen transferred between plants by bees), providing some confirmation that coffee is adapted to self-pollination; extreme outcrossing could possibly result in outbreeding depression. One explanation for the higher fruit retention in hand-pollinated branches than insect pollinated branches is possibly due to the larger amount of pollen grains successfully transferred by the former method. Huth and Pellmyr (2000) were able to determine that higher fruit retention could be obtained with higher pollen loads. The higher percent of fruit retained in the hand-pollination was not reflected in the yield (yield in hand-pollination was not significantly

different than insect exclusion); supporting the concept that out-crossing improves both bean quality and yield.

Insect pollination affected coffee bean quality as quantified by bean length, bean mass and percentage peaberry formation. In general, insect access to flowers resulted in increased bean length, increased bean mass, and reduced percent peaberry formation compared to IE. The bean length is most likely correlated with weight. Increased bean mass due to insect pollination is consistent with the study done by (Manrique and Thimann, 2002). Hand-pollination also resulted in reduced percent peaberries compared to insect-excluded flowers. The formation of peaberries can be due to poor pollination (Sybenga, 1960). In this study, percent peaberry formation in the open pollinated treatment of Year II was similar to that reported by Clifford and Willson (1985), 10-15%. Having more pollen transferred by hand-pollination in Year II resulted in only 5.4 percent peaberry formation. This supported the assertion that enhancing pollen transfer for coffee will increase coffee bean quality and reduce peaberries (Ricketts et al., 2004). It is unclear why peaberry formation was very low in Year I, but even in that year, open pollinated flowers had less peaberry formation than insect-exposed flowers.

Differences in fruit set, fruit retention, and quality between Year I and II could have been due to the overbearing of coffee fruits and lack of branch pruning in Year I. The plantation was overgrown in Year I. Over-production of coffee fruits, as a consequent of improper branch pruning is known to result in leaf loss, fruit nutrient depletion, fruit loss, and eventually stem die-back (Bittenbender and Smith, 2008). Another ramification of over production is to cause the crop for the next season to be small, resulting in biennial bearing patterns (Beaumont and Fukunaga, 1958). In Year I there was some branch die-back, which started with complete leaf necrosis and eventual loss of fruit from those branches. Both treatments lost branches due to die-back and this could have caused an unusually high rate of fruit loss. In Year II the coffee plants were pruned prior to blooming, and very few branches were lost due to die-back compared to the previous year. The mean size and mass of the beans were also different between the two years. Year II had larger beans in all treatments compared to Year I. Another difference was the number of flowers produced per node. Year I produced significantly more flowers than year two. Masarirambi et al. (2009) found that increasing irrigation

after high soil moisture depletion can increase both number of flowers produced and number of beans produced. This suggests that variable climatic conditions could be a factor for the differences seen in flower numbers and yields between both years. Along with variation in rainfall and irrigation, the size of the previous crop, pruning and fertilization can also affect yields (Masarirambi et al. 2009). These factors can also affect harvesting time and may be an explanation for the variable harvest times that occurred in both years. Although pollination can be improved with insect pollinators, pruning, weather, and overall crop health is important in improving and maintaining consistent fruit production for coffee as well.

Efficacy of Insect Pollen Transfer. Pollen transfer to the coffee stigmas was mainly affected by honeybees in this study. Transfer of pollen by a single honeybee visit was not different on stigmas exposed all day and indicated the significance of maintaining honeybee populations in coffee plantations. Currently, the study was based on 17 insect visits for stigmas exposed all day, 13 stigmas visited by one honeybee, and 9 stigmas that had no insect visitation. Factors that may account for the high variability observed in these three treatments could be the length of time each insect spent on the flower, or their behavior while foraging.

In conclusion, honeybees were the most abundant insects visiting coffee flowers and likely contributed the most to pollen transfer in this study. Overall, the results indicate that it is important to have insect visitation to increase coffee fruit set, fruit retention, bean quality, and yield. The main implication of this study is that coffee farmers would benefit from maintaining honeybee hives in coffee plantations. Despite the low abundance of syrphids, their potential as pollinators should not be ignored.

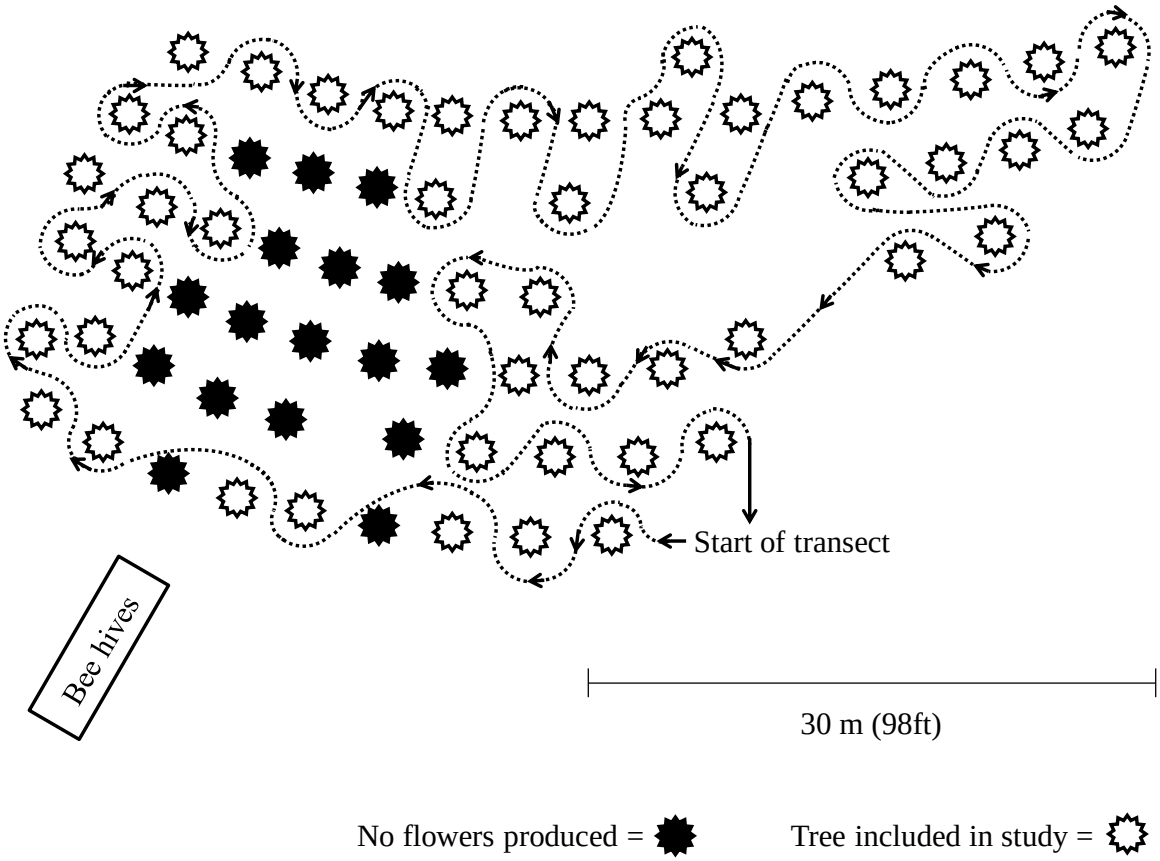

Figure 3.1. Map of coffee plantation in Waiahole, HI, showing the path followed for the insect transect observations.

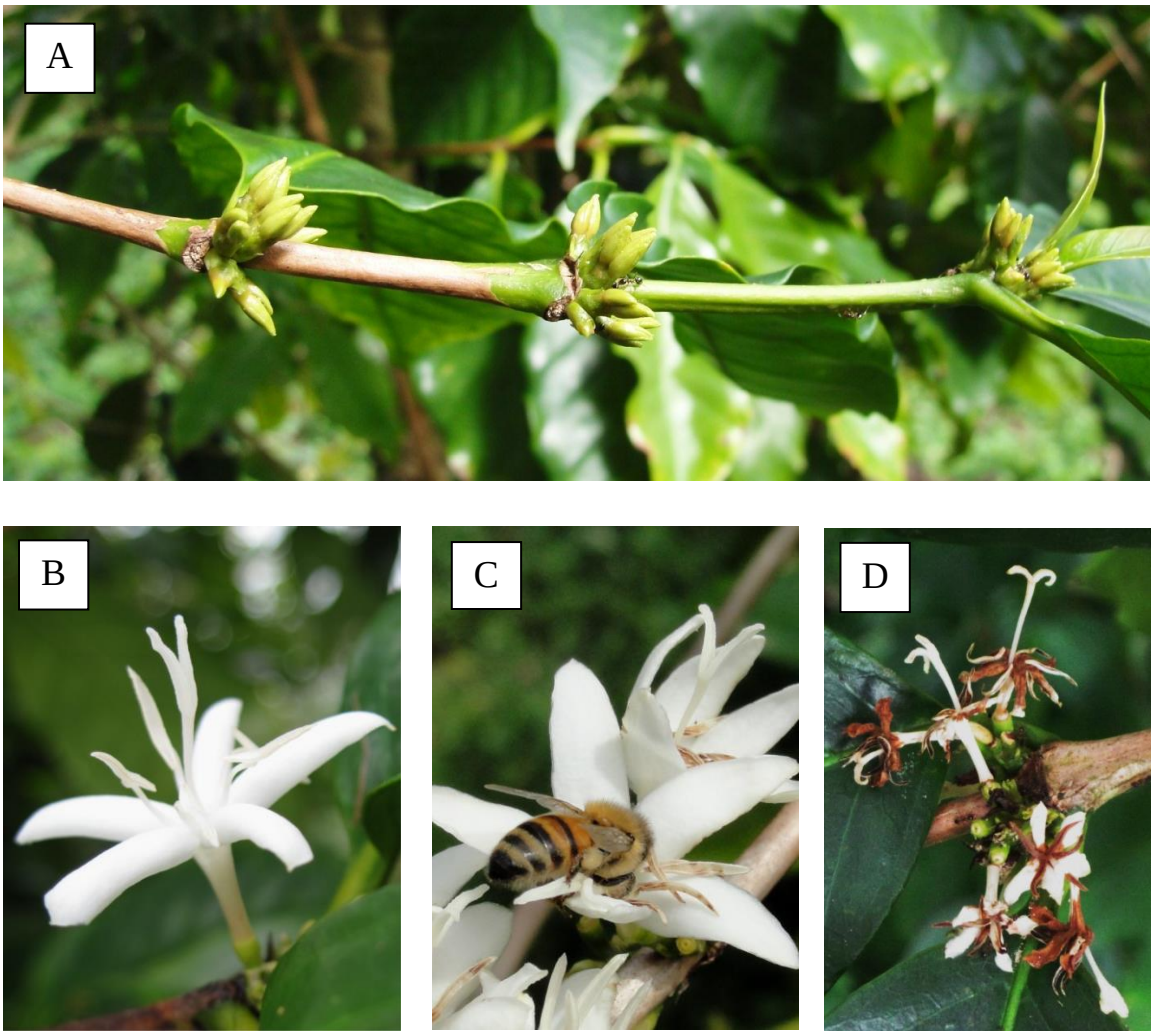

Figure 3.2. A) Three nodes on a branch showing coffee flower buds, B) coffee flower at anthesis, stigma receptive and anthers have shed pollen, C) coffee flower one day old with a honeybee; note the browning anthers, and D) coffee flowers that are three- and four days old.

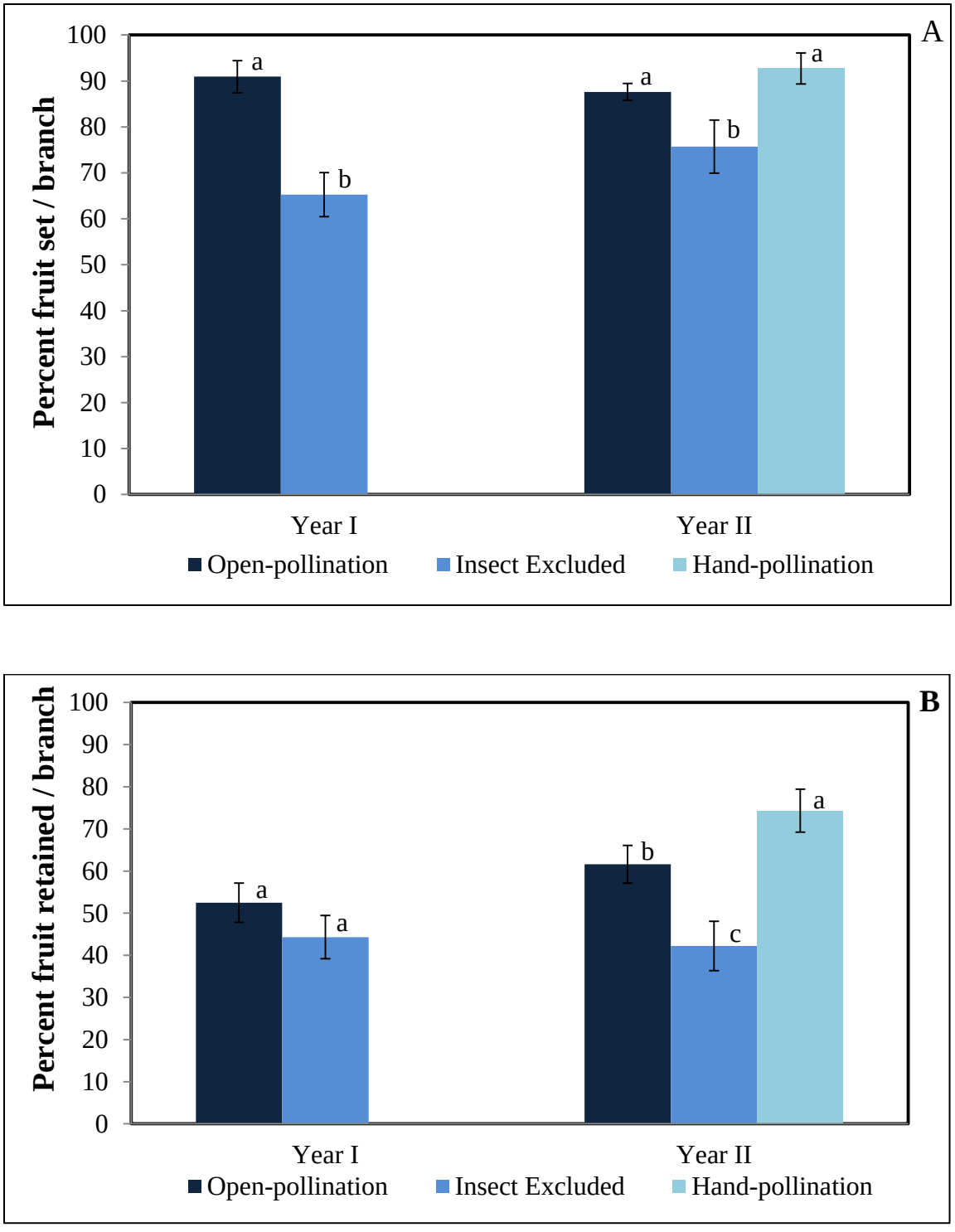

Figure 3.3. A) Initial fruit set at 21 days after flowering, and B) fruit retention at 3.5 months after flowering. Error bars shown are standard errors. Bars with different letters were significantly different ($P < 0.01$) based on Students *t*-tests and Waller-Duncan tests in Year I (n = 10) and Year II (n = 20), respectively.

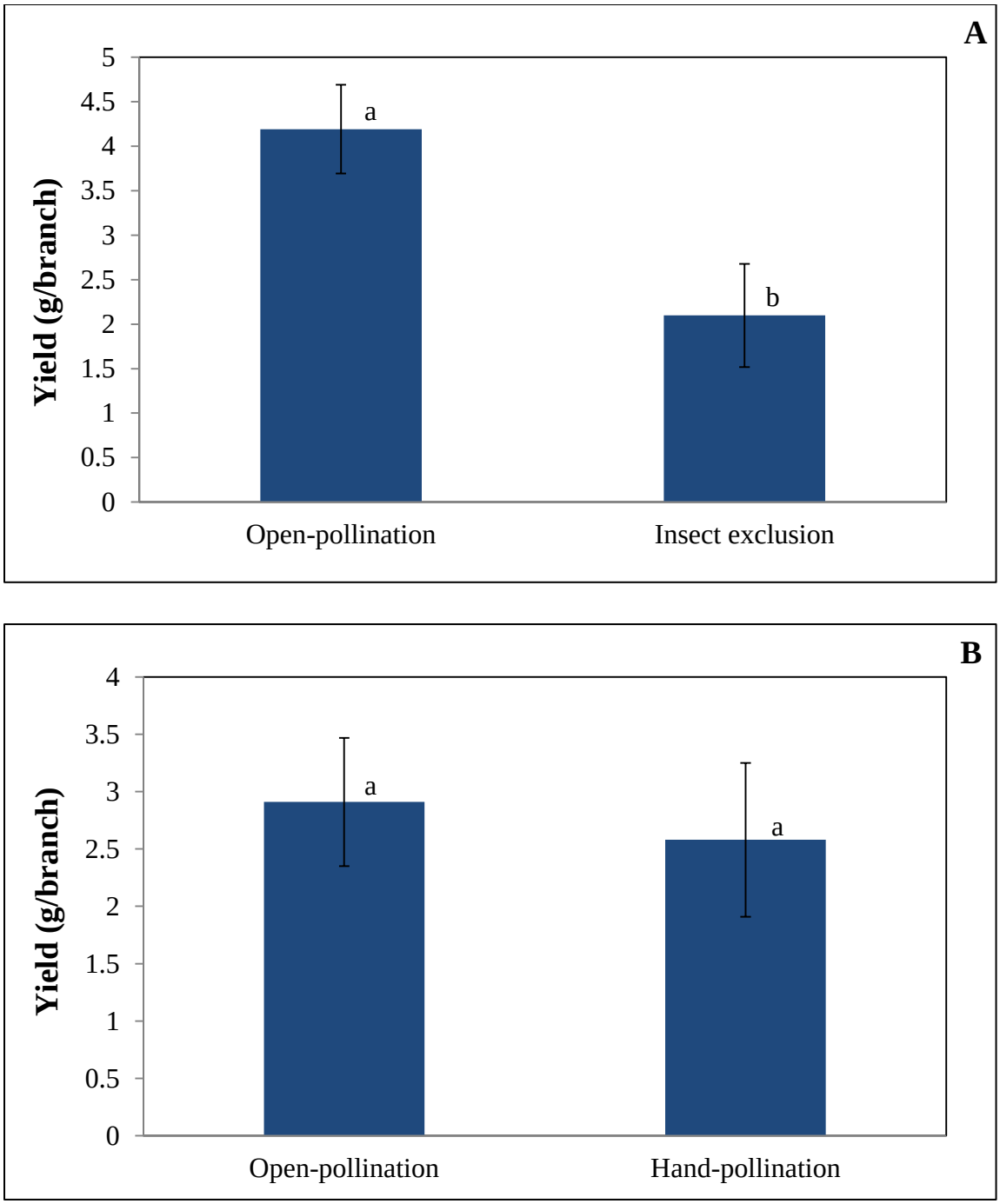

Figure 3.4. Coffee yield of A) open-pollination and insect exclusion for Year I and II, and B) open-pollination and hand-pollination for Year II. Error bars are standard errors. Bars with different letters were significantly different ($P < 0.01$) based on Students *t*-tests.

Table 3.1. Abundance of insects observed visiting coffee flowers during transect surveys Waiahole, Oahu, Hawaii

Insect Taxa		Total sightings	Abundance (%)
Hymenoptera	*Apis mellifera* (Apidae)	1041	86.3
	Xylocopa sonorina (Apidae)	3	< 1
	Pheidole sp. (Formicidae)	55	4.6
	Technomyrmex difficilis (Formicidae)	1	< 1
Diptera	*Allograpta obliqua* (Syrphidae)	67	5.6
	Ornidia obesa (Syrphidae)	4	< 1
	Syritta spp. (Syrphidae) (two different spp.)	4	< 1
	Toxomerus marginatus (Syrphidae)	4	< 1
	Eristalis arvorum (Syrphidae)	1	< 1
	Rhinia apicalis (Calliphoridae)	8	< 1
	Drosophila sp. (Drosophilidae)	4	< 1
	Hydrotaea sp. (Muscidae)	2	< 1
Lepidoptera	*Macroglossum* sp. (Sphingidae)	11	< 1
	Papilio xuthus (Papilionidae)	1	< 1

Table 3.2. Incidence of each forager type observed during flower observations on transects in Year II from 8:00 am to 4:00 pm over a period of 4 days (n = number of insects).

Insect Visitor/Forager Type	Percent forager type
Apis mellifera (n = 821)	
Nectar	21.2
Pollen	75.8
Both	3.0
Allograpta obliqua (n = 51)	
Nectar	3.9
Pollen	96.1
Pheidole sp. (n = 47)	
Nectar	95.7
Pollen	4.3

Table 3.3. Summary table including bagging protocol for treatments, sample sizes, and means (±SEM) for flower abundance

	Treatment	Bagged protocol	Sample size (# of branches)	Total flowers / treatment	Mean flowers / branch	Mean flowers / node
Year I, 2010	Open-pollination	No	8	305	38.1A[a] (±1.62)	12.2A (±0.67)
	Insect Exclusion	While flowers were attractive to insects	6	227	37.8A (±11.94)	9.5B (±0.91)
Year II, 2011	Open-pollination	Bagged for 3 weeks after flowers were not attractive to insects	18	265	14.7A (±2.33)	4.1A (±0.47)
	Insect Exclusion	While flowers were attractive to insects	18	289	16.1A (±2.67)	5.5A (±0.57)
	Hand-pollination	While flowers were attractive to insects	16	180	12.0A (±3.03)	4.1A (±0.56)

[a] Treatments with different letters within a year and column are significantly different ($P < 0.05$) based on based on Students *t*-tests and Waller-Duncan tests in Year I and Year II, respectively.

Table 3.4. Mean (±SEM) for fruit and bean parameters (mm) and mass (g) for coffee pollination experiments at Waiahole, HI.

	Year I Treatments, 2010	
	Open-pollination	Insect Exclusion
Mean fruit length	14.75A[a] (±0.09)	13.64B (±0.20)
Mean bean length	12.33A (±0.05)	11.73B (±0.12)
Mean fruit mass	1.33A (±0.03)	0.86B (±0.06)
Mean dry bean mass	0.20A (±0.003)	0.14B (±0.01)

	Year II Treatments, 2011		
	Open-Pollination	Insect Exclusion	Hand-Pollination
Mean fruit length	16.97A (±0.12)	16.59A (±0.24)	16.77A (±0.14)
Mean bean length	13.83A (±0.06)	13.51B (±0.13)	13.62AB (±0.08)
Mean fruit mass	1.75A (±0.04)	1.63A (±0.06)	1.76A (±0.05)
Mean dry bean mass	0.26A (±0.004)	0.24B (±0.007)	0.25AB (±0.005)

[a] Means are average of 10 branches in Year I and 20 branches in Year II. Treatment means with different letters in the same row were significantly different ($P < 0.05$) based on Students *t*-tests and Waller-Duncan tests in Year I and Year II, respectively.

Table 3.5. Mean (±SEM) percent and mass (g) of peaberries for both years. Total beans include both normal beans and peaberries

		Total Beans	Total pea-berries	% Peaberries / treatment	% Average peaberries / branch	Mean mass of peaberry (g) (±SEM)	Mean length of peaberry (cm) (±SEM)
Year I	OP	255	7	2.7	4.7A[a] (±2.65)	0.18A (±0.03)	11.08A (±0.28)
	IE	93	7	7.5	42.8A (±23.41)	0.12A (±0.04)	9.98A (±0.66)
Year II	OP	198	20	10.1	14.6B (±4.14)	0.29A (±0.02)	12.1A (±0.23)
	IE	88	16	18.2	34.2A (±9.98)	0.26A (±0.02)	12.1A (±0.22)
	HP	148	8	5.4	3.8B (±2.17)	0.28A (±0.02)	11.8A (±0.38)

[a] Treatment means with different letters in the same column were significantly different ($P < 0.01$) based on Students *t*-tests and Waller-Duncan tests in Year I and Year II, respectively.

Table 3.6. Comparing mean (±SEM) mass (g) of peaberries and normal beans within each treatment

	Treatment	Mean mass (g) (±SEM) for normal beans	Mean mass (g) (±SEM) for peaberries
Year I, 2010	Open-pollination	0.20A[a] (±0.06)	0.12B (±0.11)
	Insect exclusion	0.14B (±0.64)	0.18A (±0.07)
Year II, 2011	Open-pollination	0.26B (±0.05)	0.29A (±0.08)
	Insect exclusion	0.24A (±0.06)	0.27A (±0.06)
	Hand-pollination	0.25A (±0.06)	0.28A (±0.05)

[a] Treatment means with different letters in the same row were significantly different ($P < 0.05$) based on a One way analysis Students *t*-tests and Waller-Duncan tests in Year I and Year II, respectively.

Table 3.7. Inferred pollen transfer efficacy of insects on insect-exposed coffee flowers

Insect visitation opportunity	Sample #	Mean pollen grains / stigma (±SEM)	Estimated mean number of pollen grains transferred by insects
All day	17	889.7A[a] (±158.08)	591
One honeybee visit	13	658.5A (±116.01)	360
0 visit	8	298.8B (±83.71)	0

[a] Treatment means with different letters in the same column were significantly different ($P < 0.05$) data were log transformed, and Waller-Duncan tests used for pairwise comparisons. To correct for self-pollination, 298.8 was subtracted from pollen counts for insect-exposed treatment averages to estimate the amount of pollen transferred by the insect visitors.

3.6. References

Ballantyne, G., and P. Willmer. 2012. Nectar theft and floral ant-repellence: A link between nectar volume and ant-repellent traits? PLoS ONE. 7(8): e43869. doi:10.1371/ journal.pone.0043869.

Beaumont, J. H., & E. T. Fukunaga. 1958. Factors affecting the growth and yield of coffee in Kona, Hawaii. Hawaii Agricultural Experiment Station Bulletin 113. Honolulu, Hawaii.

Bittenbender, H. C. and V. E. Smith. 2008. Growing coffee in Hawaii: Revised edition. University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources http://www.ctahr.hawaii.edu/oc/freepubs/pdf/coffee08.pdf.

Clifford, M., and K. Willson. 1985. Coffee botany, biochemistry and production of beans and beverage. AVI Publishing Company, Inc. Westport, CT.

Connor, L. 2011. Fighting Back: Olivarez Honey Bees, Big Island Queens. American Bee Journal. http://www.americanbeejournal.com/site/epage/132583_828.htm.

Davis, L. E., A. G.Stephenson, and J. A. Winsor. 1987. Pollen competition improves performance and reproductive output of the common zucchini squash under field conditions. Journal of American Society for Horticultural Science. 112: 712-716.

De Jong, T. J., P. G. L. Klinkhamer and M. J. Van Staalduinen. 1992. The consequences of pollination biology for selection of mass or extended blooming. Functional Ecology. 6: 606–615.

Galen, C. 1999. Flowers and enemies: predation by nectar-thieving ants in relation to variation in floral form of an alpine wildflower, *Polemonium viscosum*. Oikos. 85: 426–434.

Ghazoul, J. 2001. Can floral repellents pre-empt potential ant-plant conflicts? Ecology Letters. 4: 295–299.

Haslett, J. R. 1989. Adult feeding by holometabolous insects: pollen and nectar as complementary nutrient sources for *Rhingia campestris* (Diptera: Syrphidae). Oecologia. 81: 361-363.

Huth, C. J., and Pellmyr, O. 2000. Pollen-mediated selective abortion in Yuccas and its consequences for the plant-pollinator mutualism. Ecology 81:1100–1107.

Klein, A.-M. 2009. Nearby rainforest promotes coffee pollination by increasing spatio-temporal stability in bee species richness. Forest Ecology and Management. 258: 1838-1845.

Klein, A.-M., Steffan-Dewenter, I., and Tscharntke, T. 2003. Fruit set of highland coffee increases with the diversity of pollinationg bees. Proceedings of the Royal Society of London. 270: 955-961.

Manrique, A. J., and R. E. Thimann. 2002. Coffee (*Coffee arabica*) Pollination with Africanized Honeybees in Venezuela. Interciencia. 27: 414-416.

Martins, D. 2008. Coffee (Coffea arabica) in forest and agro-forestry cultivation in Jimma, Ethiopia. Initial survey of good pollination practices. Food and Agriculture Organization (FAO). Rome, Italy.

Masarirambi, M. T., V. Chingwara, and V. D. Shongwe. 2009. The effects of irrigation on synchronization of coffee (*Coffea arabica* L.) flowering and berry ripening at Cipinge, Zimbabwe. Pysics and Chemistry of the Earth, Parts A/B/C. 34 (13-16): 786-789.

(NASS) National Agricultural Statistics Services. 2012. Hawaii Agriculture 2011. USDA, Hawaii Field Office. Honolulu, HI. http://www.nass.usda.gov/Statistics_by_State/Hawaii/Publications/Miscellaneous/hiag.pdf.

Ngo, H. T., A. C. Mojica, and L. Parker. 2011. Coffee plant - pollinator interactions: a review. Canadian Journal of Zoology. 89: 647-660.

Philpott, S. M., S. Uno, and J. Maldonado. 2006. The importance of ants and high-shade management to coffee pollination and fruit weight in Chiapas, Mexico. Biodiversity and Conservation. 15: 487-501.

Raw, A., and J. B. Free. 1977. The pollination of coffee (*Coffea arabica*) by honeybees. Tropical Agriculture. 54: 365-370.

Ricketts, T. H. 2004. Tropical Forest Fragments Enhance Pollinator Activity in Nearby Coffee Crops. Conservation Biology. 18: 1262-1271.

Ricketts, T. H., G. C. Daily, P.R. Ehrlich, and C. D. Michener. 2004. Economic value of tropical forest to coffee production. Proceedings of the National Academy of Sciences. 101: 12579-12582.

Roubik, D. W. 1995. Pollination of cultivated plants in the tropics, FAO Agricultural Services Bulletin 118. Food and Agriculture Organization (FAO). Rome, Italy.

Roubik, D. W. 2002. Feral african bees augment neotropical coffee yeild, pp. 255-266. *In* P. Kevan and V. L. Imperatriz Fonseca (eds.), Pollinating Bees - The Conservation Link Between Agriculture and Nature. Ministry of Environment, Brasil.

Sahil, H. F. and J. K. Conner. 2006. Characterizing ecological generalization in plant-pollination systems. Oecologia. 148: 365-72.

Sass, J. 2011. Why we need bees: Nature's tiny workers put food on out tables. Natural Resources Defense Council (NRDC). http://www.nrdc.org/wildlife/animals/files/bees.pdf.

Schneider, F. 1969. Bionomics and physiology of aphidophagous Syrphidae. Annual Review of Entomology, 14: 103-124.

Sybenga, J. 1960. Genetics and cytology of coffee: a literature review. Bibliographia Genetica. 19: 217-316.

Takashi, Y. I., and G. Kudo. 2003. Floral color change in *Weigela middendorffiana* (Caprifoliaceae): reduction of geitonogamous pollination by bumble bees. American Journal of Botany. 90: 1751-1757.

Vergara, C. H., and E. I. Badano. 2009. Pollinatior diversity increases fruit production in Mexican coffee plantations: The importance of rustic management systems. Agriculture, Ecosystems and Environment. 129: 117-123.

CHAPTER 4
EFFECT OF INSECT POLLINATION ON *DIMOCARPUS LONGAN* FRUIT PRODUCTION IN HAWAII

4.1. Abstract

A range of tropical and subtropical fruits are grown in Hawaii. Often *Apis mellifera* (the honeybee) contributes to pollination of these crops. Longan is one of the many specialty crops grown in Hawaii. However, specific details on the relative importance of *A. mellifera,* along with other potential insect pollinators that visit longan, have not been quantified in Hawaii. In this study, pollinator contributions in a longan orchard were measured by addressing: 1) species richness, and abundance of insects visiting flowers in the orchard, and 2) the effects of insect pollination in regards to fruit set, fruit retained and fruit size, weight, and percent sucrose content. The results showed that while the order Diptera was highest in species richness (14 species) of flower visitors, *A. mellifera* was the most abundant species (54.0% in abindance) vising the longan flowers. Although insects improved fruit set and retention, wind may have also contributed to pollination. Quality of fruit was significantly improved with insect pollination.

4.2. Introduction

Longan (*Dimocarpus longan*, Sapindaceae) is categorized as a tropical specialty fruit in Hawaii. In 2008 Hawaii had 70 longan farms (NASS, 2008). Longan trees require a vector, whether it is insects or wind, to facilitate pollination (Menzel and Waite, 2005; Boonithee, 1991, Blanche et al., 2006). This is due to longan being monoecious. Longan flowers are classified as three types, (Figure 4.1A, B, and C): Type I functions as male (has functional stamens that produce pollen but lack pistils), Type III are hermaphroditic but function only as male flowers (producing only pollen), and Type II are hermaphroditic but only function as female (flowers have a functional stigma and can produce fruit). Insect pollinators are integral in transferring pollen between flower types

(Menzel and Waite, 2005). Longan can be pollinated by honeybees, *Trigona* spp. (Apidae), and hoverflies (Menzel and Waite, 2005; Boonithee, 1991; Blanche et al., 2006). Research in Australia and Thailand has shown that fruit set and fruit retained are higher in *D. longan* panicles visited by insect visitors compared to insect-excluded panicles (Manning, 2006; Blanche et al., 2006). Specific objectives of this study were to examine: 1) species richness and abundance of potential pollinators visiting longan flowers, 2) the impacts of insect pollination on longan fruit quantity and quality.

4.3. Materials and Methods

4.3.1. Study Site

This study was conducted in a commercial longan orchard in the Mililani Agricultural Park (21°45' N 158°01' W) on the island of Oahu between October 2010 and April 2011. There were 65 longan trees in six rows, encompassing 38 x 130 m² and containing two cultivars; Si Chompoo and Biew Kiew (Figure 4.2). For this study potassium chlorate was applied to eight trees to induce blooming; however, only two trees, of the cultivar Biew Kiew, bloomed (indicated by a star on map, Figure 4.2). There were no managed bee hives in Mililani Agricultural Park.

4.3.2. Monitoring Insect Foragers for Species Richness and Abundance

Monitoring of insect foragers was conducted on clear sunny days, between 6:00 am to 6:00 pm when panicles were blooming. Insects were identified to species level, accumulated, and voucher specimens were deposited in the University of Hawaii Insect Museum. In addition to species richness, insect abundance of each species was also recorded between October 6 and October 26, 2010 corresponding to the synchronized blooming from Type I flowers (October 6- 20), Type II (October 11- 24) to Type III flowers (October 11 – 26). Type I, II, and III flowers were attractive to insect visitors.

4.3.3. Transect and Focal Insect Counts

Similar to the macadamia nut study (Chapter 2), two different methods were used to assess insect abundance, and the same protocol was followed. Unfortunately, only two

trees responded to the potassium chlorate intended to force blooming, so to avoid counting the same insects repeatedly, counts were made over three weeks.

The perimeter of each tree was walked during each transect. Each tree took about 11.5 minutes. About 43 panicles were observed in this time frame. During the focal observations each individual panicle was watched for 10 minutes. A total of 70 panicles were observed. For both transect and focal insect counts insects were classified as to whether they foraged for pollen, or nectar.

4.3.4. Fruit Set and Fruit Retained

To assess the impact of insect visitation on fruit production, panicles with flower buds (Figure 4.3A) averaging 1.5 meters above the ground were used. These branches were either 1) bagged and wire tied with a fine mesh cloth (measuring 71.1cm × 65.0cm), and constituted the "insect exclusion" (IE) treatment (Figure 4.3B); or 2) tagged with flagging tape but not bagged during the flower receptive stage, and constituted the "open-pollination" (OP) treatment (Figure 4.3C). A wire frame was constructed and fitted into each mesh bag to prevent damage to panicles in IE treatments. Fruit set and fruit retention on each experimental panicle was recorded throughout the development of the longan fruit; from fertilization to harvest (about 6 months). To avoid any effects of bagging on initial fruit development, OP inflorescences were also bagged once flowers were no longer attractive to insects. Each treatment was allocated to each tree used in the study. Sample size was four panicles for IE and four panicles for OP, two sub-samples per tree. Average number of flowers per panicle was 2404, with an average ratio of 5 male flowers to 1 female flower.

Fruit set was counted four weeks after female flowers had opened. Fruit retention was counted every four weeks until fruits were no longer being lost. Panicles were harvested when fruits were brown and smooth. All fruits harvested were accumulated as total yield.

4.3.5. Fruit Quality

Fruit quality was assessed by recording length, width, and mass of individual fruits at harvest. The measurements of the length and width were measured to the nearest

0.01 mm using digital calipers. These measurements included width and length of 1) the entire fruit, including exocarp, and seed (widest reading on caliper), and 2) the seed. Mass was measured to the nearest 0.01g using a digital scale. The mass recorded were 1) weight of whole fresh fruit, 2) peeled fruit and seed, and 3) seed. A sucrose Brix Refractometer was used to measure the percent sucrose for individual fruits within both treatments. A total of 44 fruits for OP and 32 fruits for IE were tested for sucrose levels.

4.3.6. Statistical Analysis

Data for fruit set, and fruit retention were subjected to analysis of variance, log transformation was done to achieve normal distribution, and means were separated using Students *t*-tests in SAS 9.2 (SAS Institute Inc. Cary, NC). Yield, and fruit quality data were subjected to analysis of variance, and means were separated using Students *t*-tests in SAS 9.2 (SAS Institute Inc. Cary, NC).

4.4. Results

Species Richness and Abundance of Insect Visitors. Thirty-three species of insects belonging to five orders (Coleoptera, Diptera, Hymenoptera, Lepidoptera, and Mantodea) were observed on longan flowers (Table 4.1, 4.2). The number of species recorded within each order included 5 Coleoptera, 14 Diptera, 11 Hymenoptera, 2 Lepidoptera, and 1 Mantodea. During transect and focal analysis (Table 4.1. 4.2), *A. mellifera* was the most abundant species, and accounted for 54.0%, and 50.4% of all the insect visitors respectively. Although many insects were scarce (<1%) during transect and focal analyses, the order Diptera accounted for 40.8% and 45.6% of all insect visitors respectively. Nine insect species recorded during transect insect counts were not observed during the focal insect counts, and 3 species recorded during focal insect counts were not observed during the transect counts.

Longan flowers are small and with many of the insects it was difficult to distinguish the foraging behavior. Honeybees and the syrphid, *Allograpta obliqua,* forager types were easiest to determine. Table 4.3 shows the foraging preferences for these two insects seen during transect and focal insect counts. Nectar was preferred to pollen for the honeybee and for *A. obliqua,* pollen was preferred to nectar.

Impacts of Insect Pollination on Longan Fruit Set, Fruit Retention, Fruit Quality, and Yield. Although OP produced significantly more fruit set per panicle (4 times higher) compared to IE ($F_{1,3} = 18.99$; $P < 0.05$), the fruit retained at harvest (22.2% more fruit) compared to IE was not significant ($F_{1,1} = 0.29$; $P > 0.05$, Figure 4.4A, B). For this study fruit abortion stopped 3.5 months after female flowers had bloomed. This could be verified by same number of fruits harvested at the end of both trials to the number of fruits counted at this last abortion date. Panicles were harvested in April 2011.

In terms of quality, open-pollination had heavier fruits ($F_{1,74} = 4.2$; $P < 0.05$), larger seeds ($F_{1,74} = 12.45$; $P < 0.001$), heavier seeds ($F_{1,121} = 17.0$; $P < 0.0001$), and had higher percent sucrose levels ($F_{1,74} = 4.8$; $P < 0.05$, Table 4.4).There was no significant difference in the other quality parameters ($P > 0.05$). Panicles exposed to insect pollination expressed higher fruit sets regardless of what stage the fruit was at in its development; however, there was no significant difference for yield per panicle between OP and IE ($F_{1,2} = 0.51$; $P > 0.05$, Table 4.4).

4.5. Discussion

Species Richness and Abundance of Insect Visitors. Based on this study Diptera contributed the most species of flower visitors on longan flowers, while the species with highest abundance was *A. mellifera.* Although most of the Diptera were in lower abundance compared to *A. mellifera*, the high abundance of Diptera as a whole could have also contributed to pollination. Menzel and Waite (2005) found that litchi, which is in the same family as longan and has similar flower biology, had an increase in fruit set and fruit retention with both honeybee and syrphid pollinators. Although the syrphids were less abundant compared to honeybees in the study orchard, they may have contributed to pollination.

Hylaeus strenuus, an adventive bee species, was seen foraging on longan flowers. Presently, records of this insect have been constrained to the lowlands east of Honolulu, where it has been found infrequently (Magnacca et al., 2010). During this study, this insect was not only seen in the orchard in Mililani on longan flowers but also in the surrounding areas and within the macadamia orchard at the Waimanalo Research Station,

foraging on flowers of *Sesbania grandiflora* (West Indian pea), *Asystasia gangetica* (Chinese violet) and *Mimosa pudica* (Sensitive plant).

The majority of the insects seen visiting longan flowers were present in very small numbers. The transect analysis probably increased the likelihood to observe scarce insects; however, both methods used had generally similar trends for abundance and forager type.

Compared to the macadamia and coffee experiments, overall insect visitation was high during this study (average of 22 insects seen per 10 minutes); however, wind can also facilitate pollination in longan (Smith et al., 2004; Davenport and Stern, 2005). The foliage of the longan trees in this orchard was very dense and although weather conditions were occasionally windy in this orchard during the insect abundance counts, the wind currents were extremely reduced near trees that were closely surrounded by other longan trees. Two of the panicles in the IE treatment that had no fruit at harvest were located on a tree that was entirely encompassed by other longan, blocking windy weather. However, the panicles within the IE treatment that did retain fruit until harvest were those that were located on the tree that was not closely surrounded by other longan trees and therefore exposed to wind.

Longan Fruit Set, Retention, Yield and Quality. Research has shown that insect visitation is essential for longan fruit production (Manning, 2006; Blanche et al., 2006). Our results support that insect visitation is important for pollination of longan in Hawaii and that wind pollination may have been a factor in this study. Longan can self-pollinate to a degree (Blanche et al., 2006), but to achieve this it needs a vector. The overlapping of flower types within a panicle plus wind can facilitate pollen movement from male to female flowers and may account for the fruit set in the IE treatment. Higher quality of fruit was obtained with insect polliation, confirming the importance of insect pollination. However, due to only two trees flowering for this experiment, it would be important to increase the sample size to confirm these findings. The small sample size may have also affected the results for the fruit retained and yield.

In conclusion, the honeybee was the most abundant insect and likely contributed the most to pollination. The syrphids, along with some of the other flies, may have also contributed to pollination as well.

Figure 4.1. A) Type I flower, male (flowered first), B) Type II flower, female (flowered second), and C)Type III flower, male (flowered last).

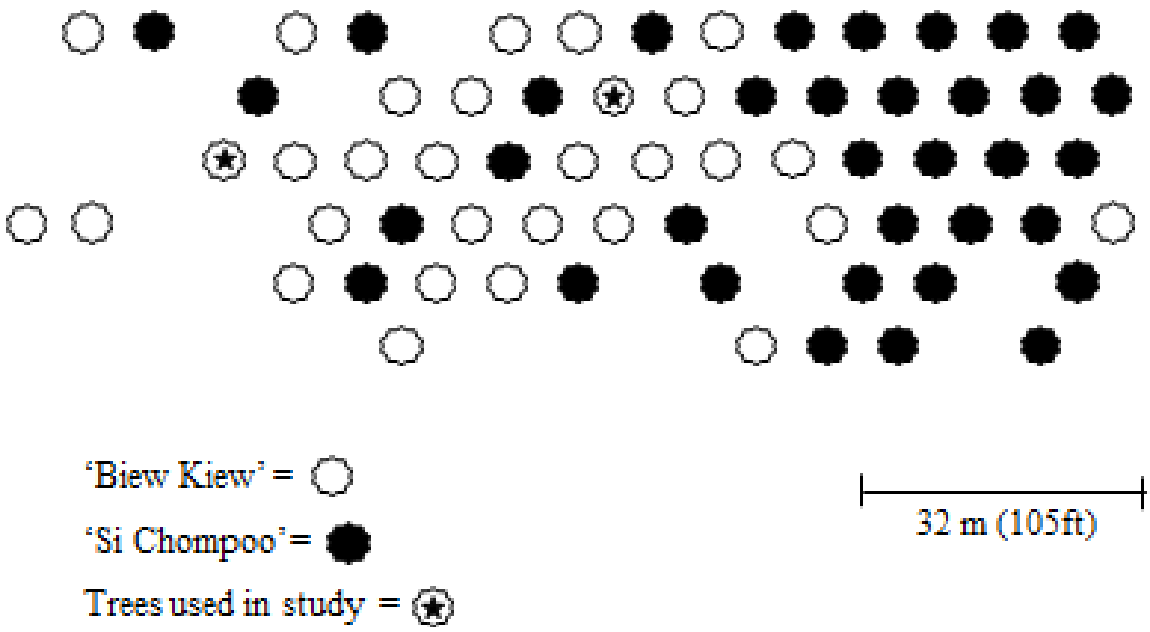

Figure 4.2. Map of orchard at the Mililani Agricultural Park showing trees used in study.

Figure 4.3. A) Immature longan panicle, B) Bagged panicle prior to blooming (IE), with wire frame sewn inside to protect flower buds, and C) OP panicle blooming.

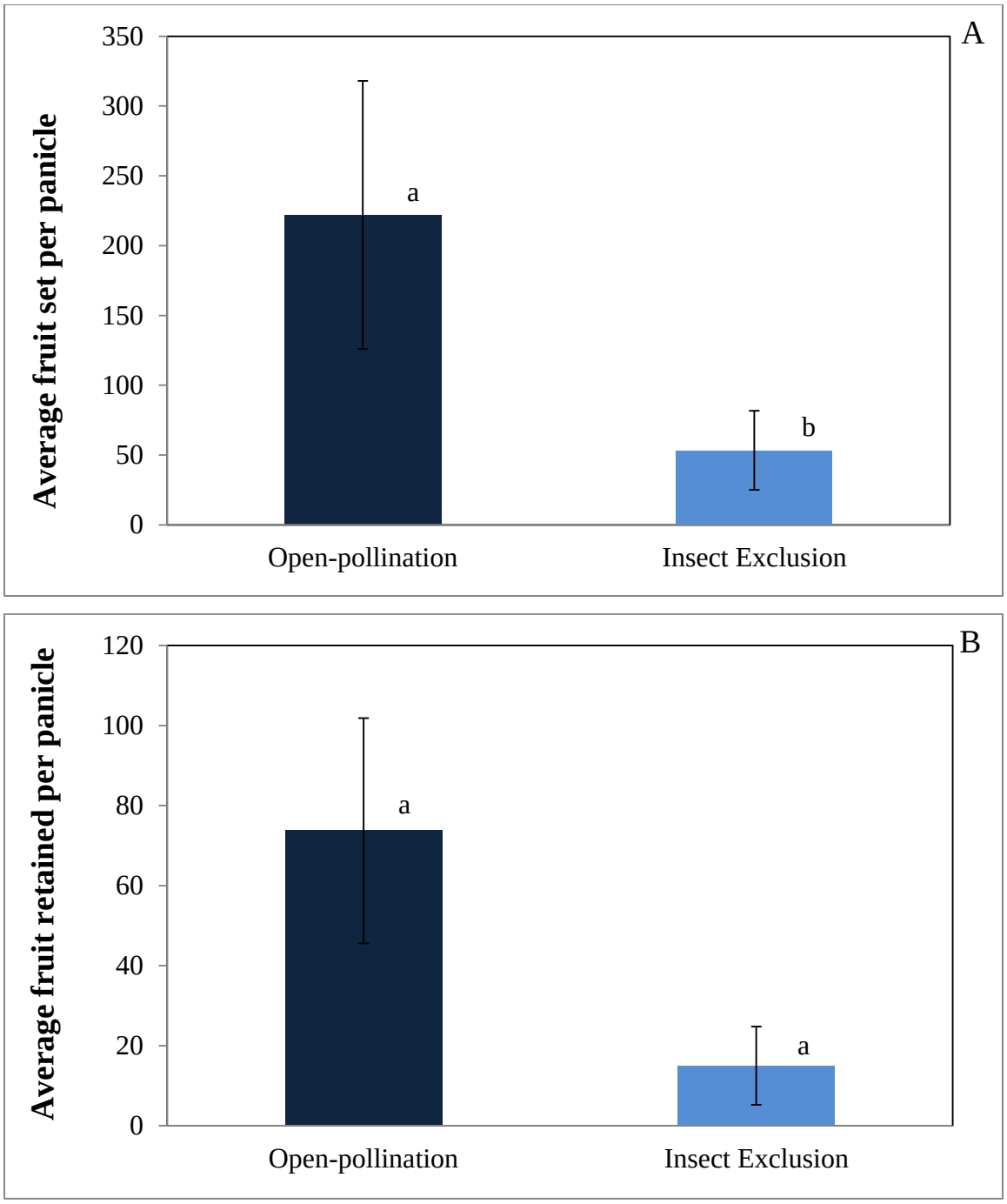

Figure 4.4. A) Initial fruit set at four weeks after flowering, and B) retained fruit at 3.5 months after flowering. Bars shown on each column are standard error bars. Bars with different letters were significantly different ($P < 0.05$), data was log transformed and means were separated using Students *t*-tests.

Table 4.1. Insect abundance on longan panicles observed during transect analysis in a longan orchard at the Mililani Agricultural Park

Order	Name	Total sightings	Abundance (%)
Hymenoptera	*Apis mellifera* (Apidae)	1836	54.0
	Xylocopa sonorina (Apidae)	78	2.3
	Lasioglossum impavidum (Halictidae)	21	< 1
	Campsomeris marginella modesta (Scoliidae)	7	< 1
	Eupelmus sp. (Eupelmidae)	4	< 1
	Megachile timberlakei (Megachilidae)	2	< 1
	Hylaeus strenuus (Colletidae)	1	< 1
	Agapostemon sp (Halictidae)	1	< 1
	Anthocephalus apicalis (Chalcididae)	1	< 1
	Apanteles sp (Braconidae)	1	< 1
	Pheidole megacephala (Formicidae)	107	3.1
Diptera	*Allograpta obliqua* (Syrphidae)	292	8.6
	Eristalis arvorum (Syrphidae)	12	< 1
	Ornidia obesa (Syrphidae)	65	1.9
	Syritta orientalis (Syrphidae)	2	< 1
	Drosophila spp. (Drosophilidae)	454	13.3
	Unknown (Muscidae)	376	11.1
	Atherigona sp. (Muscidae)	169	5.0
	Musca domestica (Muscidae)	1	< 1
	Lonchaeidae metatarsata (Lonchaeidae)	24	< 1
	Unknown	25	< 1
	Physiphora clausa (Otitidae)	12	< 1
	Trichopoda pilipes (Tachinidae)	8	< 1
	Atrichopogon jacobsoni (Ceratopogonidae)	1	< 1
	Rhinia apicalis (Calliphoridae)	0	-
Coleoptera	*Protaetia orientalis* (Scarabaeidae)	28	< 1
	Diachus auratus (Chrysomelidae)	7	< 1
	Orchidophilus aterrimus (Curculionidae)	2	< 1
	Scymnus sp. (Coccinellidae)	1	< 1
	Melanoxanthus melanocephalus (Elateridae)	0	-
Lepidoptera	*Danaus plexippus* (Nymphalidae)	2	< 1
	Hylephila phyleus (Hesperiidae)	0	-
Mantodea	*Tenodera aridifolia sinensis* (Mantidae)	3	< 1

Table 4.2. Insect abundance on longan panicles observed during focal analysis in a longan orchard at the Mililani Agricultural Park

Order	Name	Total sightings	Abundance (%)
Hymenoptera	*Apis mellifera* (Apidae)	755	50.4
	Xylocopa sonorina (Apidae)	16	1.1
	Lasioglossum impavidum (Halictidae)	5	< 1
	Eupelmus sp. (Eupelimdae)	3	< 1
	Campsomeris marginella modesta (Scoliidae)	1	< 1
	Apanteles sp (Braconidae)	1	< 1
	Megachile timberlakei (Megachilidae)	1	< 1
	Hylaeus strenuus (Colletidae)	0	-
	Agapostemon sp (Halictidae)	0	-
	Anthocephalus apicalis (Chalcididae)	0	-
	Pheidole megacephala (Formicidae)	21	1.4
Diptera	*Allograpta obliqua* (Syrphidae)	68	4.5
	Ornidia obesa (Syrphidae)	19	1.3
	Eristalis arvorum (Syrphidae)	1	< 1
	Syritta orientalis (Syrphidae)	0	-
	Drosophila spp.	396	26.5
	Atherigona sp. (Muscidae)	130	8.7
	Musca domestica (Muscidae)	12	< 1
	Unknown (Muscidae)	10	< 1
	Lonchaeidae metatarsata (Lonchaeidae)	21	1.4
	Unknown	10	< 1
	Physiphora clausa (Otitidae)	3	<1
	Rhinia apicalis (Calliphoridae)	1	<1
	Atrichopogon jacobsoni (Ceratopogonidae)	0	-
	Trichopoda pilipes (Tachinidae)	0	-
Coleoptera	*Diachus auratus* (Chrysomelidae)	7	< 1
	Protaetia orientalis (Scarabaeidae)	1	< 1
	Melanoxanthus melanocephalus (Elateridae)	1	< 1
	Scymnus sp. (Coccinellidae)	1	< 1
	Orchidophilus aterrimus (Curculionidae)	0	-
Lepidoptera	*Hylephila phyleus* (Hesperiidae)	1	< 1
	Danaus plexippus (Nymphalidae)	0	-
Mantodea	*Tenodera aridifolia sinensis* (Mantidae)	0	-

Table 4.3. Percentage incidence for each forager type observed during transect and focal analyses from 6:00 am – 6:00 pm (n = number of insects) in a longan orchard, Mililani Agr Park

Insect Visitor/Forager Type	Transect Analysis	Focal Analysis
Apis mellifera	(n = 1836)	(n = 755)
Nectar	95.0	82.8
Pollen	5.0	16.9
Both	0.0	0.3
Allograpta obliqua	(n = 292)	(n = 66)
Nectar	98.6	92.4
Pollen	1.4	7.6

Table 4.4. Comparing mean (±SEM) of longan fruit and seed parameters (mm), mass (g), and sucrose (%) for longan with and without insect access at Mililani Agricultural Park, HI.

	Open-pollination	Insect Exclusion
Mean whole fruit width	23.18A [a] (± 0.66)	22.72A (±0.32)
Mean whole fruit length	20.17A (±0.57)	18.99A (±0.28)
Mean whole fruit weight	5.49A (±0.31)	5.07A (±0.28)
Mean peeled fruit weight	5.49A (0.48)	4.25B (±0.27)
Mean seed width	9.08A (±0.62)	6.37B (±0.27)
Mean seed length	10.04A (±0.38)	9.23A (±0.38)
Mean seed weight	0.72A (±0.08)	0.23B (±0.04)
Sucrose	17.61A (±0.41)	16.29B (±0.42)
Yield (g/panicle)	228.2A (±151.05)	69.3A (±69.33)

[a] Means are an average of 4 panicles. Treatment means with different letters in the same row were significantly different ($P < 0.05$) based on Students *t*-tests.

4.6. References

Blanche, R. K., J. A. Ludwig, and S. A.Cunningham. 2006. Proximity to rainforest enhances pollination and fruit set in orchards. Journal of Applied Ecology. 43: 1182-1187.

Boonithee, A., N, Juntawong, H. Pechhacker, and E. Huttinger. 1991. Floral visits to select crops by four *Apis* species and *Trigona* species in Thailand. Acta Horticulturae. 288: 74-80.

Davenport, T. L., and R. A. Stern. 2005. Flowering, pp. 87-113. *In* C. M. Menzel and G. K. Waite, Litchi and Longan: Botany, Production and Uses. CAB International, United Kingdom.

Magnacca, K. N., W. T. Nagamine, and H.H. Dathe. 2011. *Hylaeus strenuus* (Hymenoptera: Colletidae), a new alien bee from Oahu. Bishop Museum Occasional Papers.109: 23-24.

Manning, R. 2006. Bee pollination benefits for tropical fruit crops. Department of Agriculture and Food: Government of Western Australia. http://www.agric. wa.gov.au.

Menzel, C., and G. K. Waite. 2005. Litchi and Longan: Botany, Cultivation and Uses. CABI Publishing.

Smith, N., S. A. Mori, A. Henderson, D. W. Stevenson, and S. V. Heald. 2004. Flowering Plants of the Neotropics. Princeton University Press. Princeton, NJ.

(NASS) National Agricultural Statistics Services. 2008. USDA, Hawaii tropical Specialty Fruits. http://www.nass.usda.gov/Statistics_by_State/Hawaii/.../Fruits_and.../trop frt.pdf.