Contribution of Insect Pollination
to *Macadamia integrifolia* Production in Hawaii

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Abstract. The honeybee, *Apis mellifera,* is commonly 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 parameters 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 showed 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, 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 (Syrphidae) observed in the orchard may have contributed to pollination, but likely to a lesser extent than the honeybees due to the low abundance of the flies.

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 with a connection between farmers and the local beekeepers. The growers of large macadamia orchards have long 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, *A. mellifera*, to the macadamia has not been quantified. In the family Proteaceae, pollination may be complex, with various pollinators being key for different species, e.g. insects, birds, rodents and wind may all be significant pollen vectors (Rourke and Delbert 1977, Hattingh and Giliomee 1989, Coetzee and Giliomee 1985, Wright et al. 1991, Roubik 1995), and it is thus relevant to quantify the

relative importance of insect pollinators 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, causing probably in excess of 90% reduction in feral colonies. There are no previous studies of the density of insect pollinators required to provide effective fruit set in macadamia nut orchards, because feral bees could simply augment pollination by managed hives. Understanding the importance of honeybees in macadamia orchards is underscored because of the recent impacts to bees in the state.

Previous research on macadamia nuts has highlighted several significant 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 a key 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 variable influenced by pollen transfer. The movement of pollen among plants is a key factor, with crops that need or benefit from cross-pollination. Urata (1954) reported that Syrphidae and honeybees were the most frequently observed flower visitors on macadamia, but did not quantify their role in pollination.

It is essential to account for various factors 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). The macadamia stigma is covered in pollen when it opens. However, to improve chances of cross-pollination it is necessary that the pollen be removed from a newly emerged stigma (rather than from anthers, as is the case in most plants). Assessing the ability of insects to remove pollen from the stigma is thus critical. This paper focuses on evaluating the contribution of insect pollination on macadamia nuts production in Hawaii. Specific objectives of this study were to examine: (1) species richness and abundance of potential insect 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.

Materials and Methods

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), 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 'Keaau 660' plants were dispersed as every third tree within every third row (Figure 1). 'Kau 344' 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
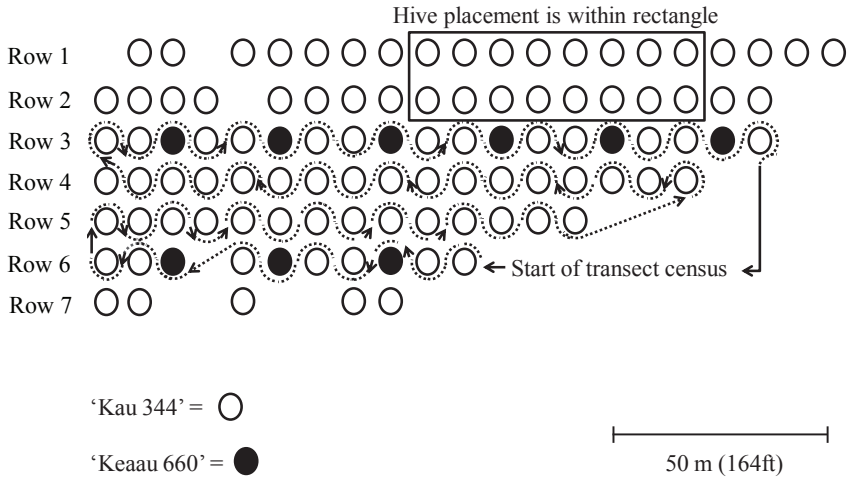
Hive placement is within rectangle

Row 1 ○ ○   ○ ○ ○ ○ ○ ○│○ ○ ○ ○ ○ ○ ○ ○ ○│○ ○ ○ ○
Row 2 ○ ○ ○ ○   ○ ○ ○ ○│○ ○ ○ ○ ○ ○ ○ ○ ○│○ ○ ○
Row 3 ○ ○ ● ● ○ ○ ● ○ ○ ● ○ ○ ● ○ ○ ● ○ ○ ● ○ ○ ● ○
Row 4 ○
Row 5 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○
Row 6 ○ ○ ● ○ ○ ● ○ ○ ● ○ ○ ● ○ ○ ← Start of transect census ←
Row 7 ○ ○   ○   ○ ○

'Kau 344' = ○

'Keaau 660' = ●

|—————————|
50 m (164ft)

Figure 1. Map of orchard at the University of Hawaii Waimanalo Research Station showing transect route.

first and second rows.

Monitoring insect foragers for species richness and abundance. Monitoring of insect foragers was conducted on clear sunny days, between 6:00 a.m. and 5:00 p.m. when trees were in bloom in both seasons. Assessment of species richness (the total number of different species) 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 flower visitor diversity, honeybee abundance was also recorded in Year I. In Year II, after becoming familiarized with the insect visitors during Year I, data on diversity and abundance of all insect pollinators was gathered.

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 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 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 mouthparts 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 3 B). On the second day of flowering, florets remain attractive to insects, still producing nectar (Figure 3 C), but by day three petals darken to tan (Figure 3 D) becoming less attractive to insects. By day four petals turn brown and will soon drop (Figure 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 bee's pollen baskets and the foraging location of the bee, coupled with the
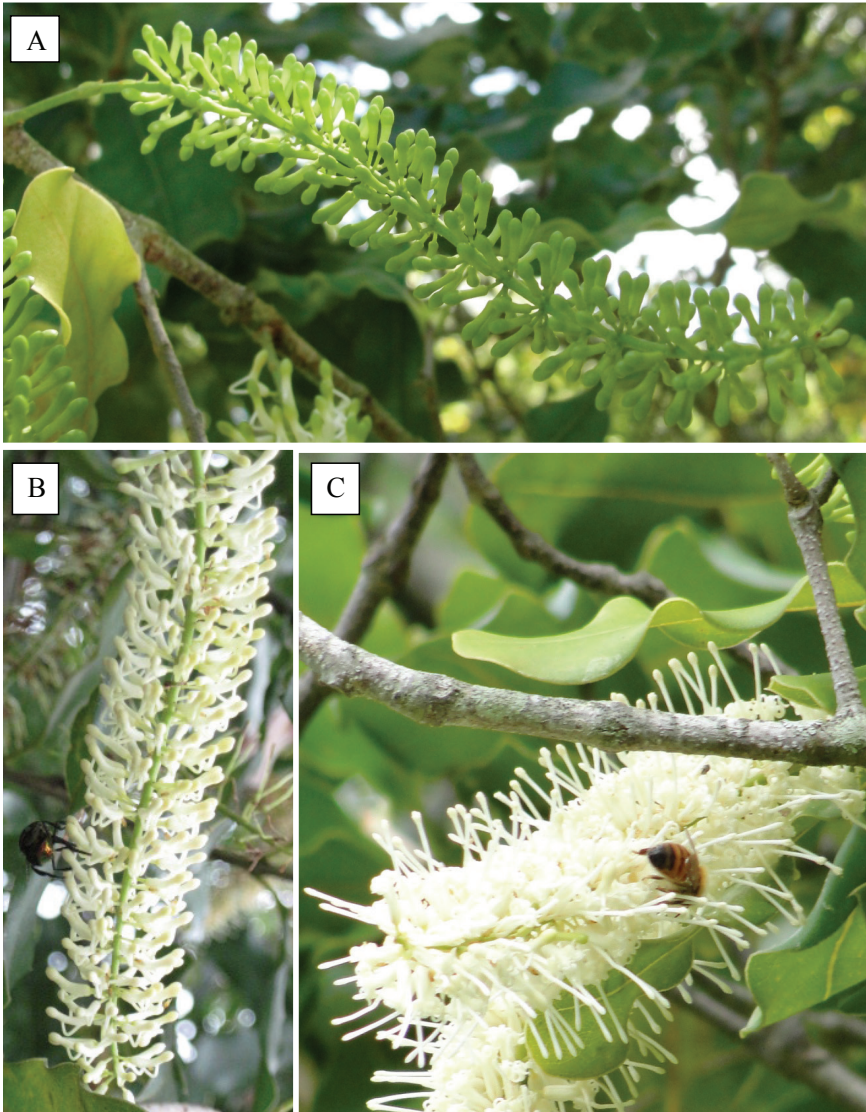
Figure 2. (A) Immature macadamia inflorescence, (B) macadamia inflorescence one day before opening with a syrphid, *Ornidia obesa,* and (C) macadamia inflorescence that has flowers completely open with a honeybee, *Apis mellifera*.

position of the pollen bearing structures in each flower. With flies, the determination of pollen or nectar foraging was made on the foraging location of the insect alone.

Transect and focal insect counts. Two different methods were used to assess in-

sect abundance: (1) transect insect count, where the observer walked briskly down the orchard rows and identified and recorded insects visiting inflorescences, and (2) focal insect count, where individual inflorescences were observed continually
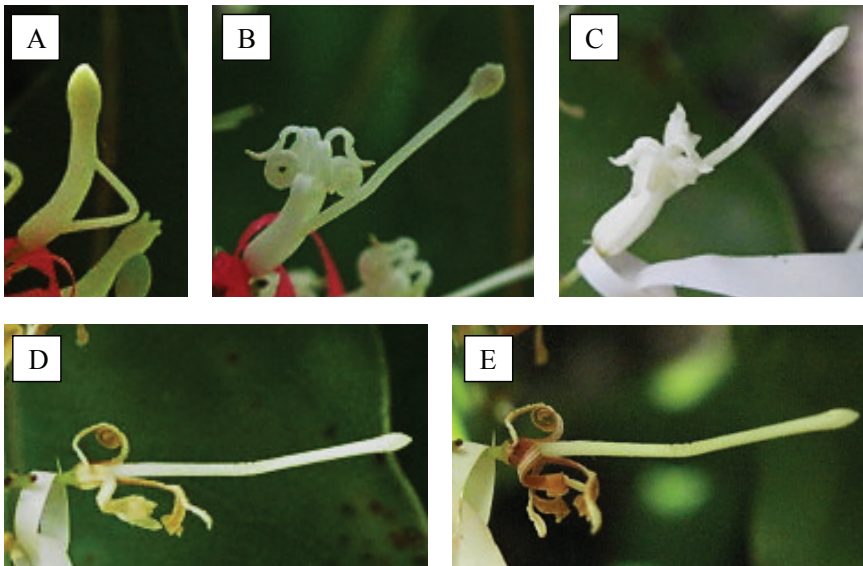
Figure 3. Progression of a macadamia flower: (A) macadamia flower one day before opening with its pistil beginning to emerge from perianth and creating a loop, (B) macadamia flower that has just opened, note pollen on the stigma, (C) two days old, note stigma is free of pollen, (D) three days old, and (E) four days old, petaloid sepals have turned brown and will soon drop off. Stigma is receptive around 2–3 days.

for 20 minutes and all insects seen visiting the inflorescence were recorded.

A fixed route was walked for the transect (Figure 1) and repeated three times in a continuous loop. All trees in the orchard were included in the study except those located directly 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, individual inflorescences were observed for 20 minutes each. For these observations, inflorescences up to 2.6 m above ground were observed. 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 classified as foraging for pollen or nectar.

Whether insects foraged on flowers that had only the style emerging from the perianth (only nectar accessible) or on flowers that were completely open (pollen and nectar accessible) was also recorded.

Fruit set and fruit retention. To assess the impact of insect visitation on macadamia nut production in both years, inflorescences at bud stage (Figure 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

71.1 cm x 54.6 cm) covering about 45.7 linear cm of branch, encompassing the conflorescence, and secured with a wire tie, which constituted the "insect exclusion" (IE) treatment; or (2) tagged with flagging tape to facilitate identification of controls over the course of the experiment, 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) blooming at least a month after trees had begun blooming (blooming is initially light and sporadic early in the season), when trees were in full bloom, and (2) located at the outside 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.

In Year I, three inflorescences per tree, with a total of eight trees, were measured to the nearest millimeter to estimate mean inflorescence length. The number of florets per inflorescence was also recorded for each inflorescence. These measurements were taken to determine the variability in inflorescence length and mean 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 unintended pruning of experimental trees during regular orchard management.

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.

Nut quality. Nut quality was assessed by recording length, width, and mass of individual nuts at harvest. Nuts sampled during both years 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) kernel in shell, with the husk removed, and (2) individual shelled kernels. 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 shelled kernels. For Year II, nuts were dried then weighed for final dry-weight values.

Efficacy of insect pollen removal. 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 collected by an insect. Potential insect ability to remove pollen from the stigma was quantified. Numbers of pollen grains on the stigma of newly open flow-

ers were counted: (1) after one insect visit (foraging for nectar or pollen, honeybees and *O. obesa*), (2) after the floret was exposed for a full day to insect visitation, or (3) with no insect visitation (insects excluded). 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 stigmas 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 4. 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 13,000 rpm for two minutes. Supernatant liquid was decanted and the gelatin with imbedded pollen was removed using a fine hook, 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 mm² grid on its surface and place under a compound microscope for counting. Each cover-slip was divided into sixteen squares and a single 1 mm² in the center of each of the sixteen squares was counted and the number of pollen grains was estimated for each sample

Statistical analysis. Means from open-pollination and insect exclusion in Years I and II 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 (PROC MIXED, SAS 9.2), and means were separated using Waller-Duncan pairwise comparisons in SAS 9.2 (SAS Institute Inc. Cary, NC).

Results
Richness and abundance of insect visitors. Thirteen species of insects belong-

ing to four orders (Diptera, Hymenoptera, Lepidoptera, and Coleoptera) were found foraging on macadamia inflorescences (Table 2). Among the Diptera were three species in Syrphidae (hoverflies), two species in Ceratopogonidae (midges), one species each in Muscidae, Milichiidae, Calliphoridae, and Chloropidae. Among the Hymenoptera, two of the species recorded were *A. mellifera*, and *Xylocopa sonorina* in Apidae. Among the Lepidoptera, one species *Tmolus echion* (larger lantana butterfly), and among the Coleoptera, one 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*.

Observations of macadamia inflorescences revealed that *A. mellifera* was the most frequent insect visitor, accounting for 62.7% of all transect observations and 46.2% of the focal observations (Table 1). It is however noteworthy that the two types of observations yielded different abundance frequencies, not only for honeybees, but also for other insect visitors. Transect data suggests that the second most abundant species on macadamia inflorescences was *Ornidia obesa* (Syrphidae), accounting for 21.2% of all the insect visits. *O. obesa* is large species of fly, with a distinctive flight and coloration. In comparison, the focal observations indicate that a small bodied fly, *Conioscinella formosa* (Chloropidae) was as abundant as honeybees on macadamia inflorescences (Table 1). In addition, four insect species recorded during transect insect counts were not recorded during focal observations.

Insect forager type (pollen, nectar, or both pollen and nectar) was recorded during focal and transect counts (Table 2). *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. Insects were preferentially foraging for nectar rather than 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.

During focal insect counts, observations were also made of insects that had a tendency to foraging on flowers that had the style emerging from the perianth but the stigma and pollen had not emerged. Thus 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 flowering stages (honeybees: 35.1%; Syrphidae [*O. obesa*]: 30.8% of individuals foraged on open flowers and flowers with pistil emerging).

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, 0.17% and 0.14% 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 (OP) consistently produced higher fruit set and fruit retained in both years (Figure 4 A, B). For Year 1, OP had three times higher fruit set than insect-excluded (IE) inflorescences 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
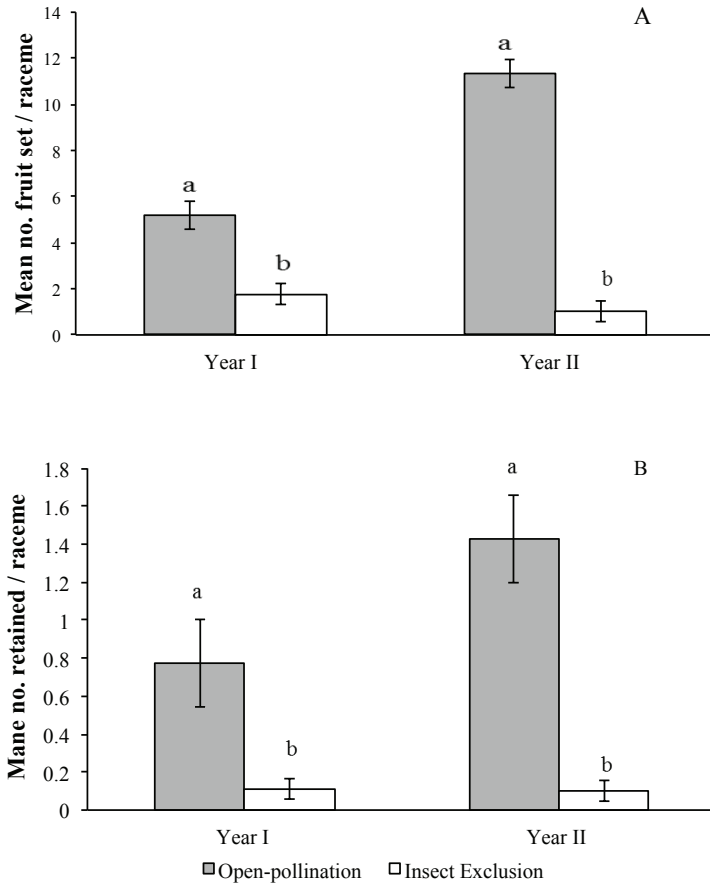
Figure 4. (A) Initial fruit set per raceme at 14 days after flowering, and (B) fruit retention at 2 months (2010, n = 10) and 3 months (2011, n = 40) after flowering (means ± SE). Bars with different letters (within a year) were significantly different ($P < 0.001$), Students t-tests.

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 retention 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 a significant difference for yields per branch between OP and IE ($P < 0.0001$, Figure 5). 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 3). However, in Year II,
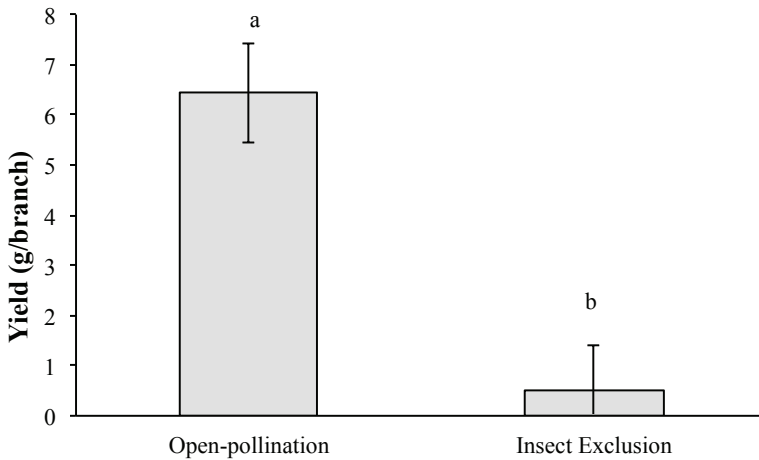
Figure 5. Mean (± SE) macadamia nut yield for open-pollination and insect exclusion treatments for year I and II combined. Bars with different letters were significantly different (*P* < 0.0001) Students *t*-test.

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 at the stage the assessments were done. 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 *O. obesa* (Syrphidae) were the predominant flower visitors, and thus were the only insects reported in Table 4. Without insect visitation, an open flower had a mean of 15,298 (±189.9) pollen grains on a stigma. Stigmas exposed to insects for a full day, and those with a single visit permitted 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 (*O. obesa* and the honeybee) was not significantly different compared to stigmas that had no insect visitation (*P* > 0.05).

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 relatively 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

Table 1. Abundance of insect visitors observed visiting macadamia inflorescences during Year II transect and focal counts at the University of Hawaii Waimanalo Research Station, Oahu, Hawaii.

Insect taxa	Transect		Focal	
	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. 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.

Analysis: Year:	Transect Year I	Transect Year II	Focal Year III
Insect species (no.) Forage type		Percent forager type	
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	-

had all insects excluded. Heard and Exley (1994) found that smaller insects have a negligible 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* was a small insect (1.5 mm) 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 central 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 substantial contributor to pollination of macadamia in the study orchard.

Although the two methods used to measure insect abundance showed similar trends in insect visitation, some of the insects were differentially detected by each method. 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 observed foraging on flowers of different flower stages. 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 mouthparts 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 key 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 little or no time 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. A foraging honeybee is not foraging just for herself, but for the 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,

Table 3. Mean (±SEM) for fruit and kernel parameters (mm) and mass (g) for macadamia pollination experiments at University of Hawaii Waimanalo Research Station.

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

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

Table 4. Pollen removal rate of insects on insect exposed macadamia flowers.

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

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

Allen et al. 2005). Yields were considerably higher in inflorescences pollinated by insects than those from which insects were excluded. It is clear that insects were important for increasing macadamia nut yields. However, in terms of quality, nut size and individual kernel mass were 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, consistent with previous findings (Urata 1954). 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 had minimal effect on pollen removal, and amounts of pollen on those flowers visited by nectar foragers compared to stigmas that received no insect visitation was similar. The small 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, as multiple visits occur for nectar foraging and therefore the chances for cross-pollination to occur would be increased.

Heard (1994) concluded that insects foraging mostly for pollen among macadamia inflorescences could 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 either pollen or 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 essential 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.

Literature Cited

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

Allen, J., J. Wilkie, C. Barnes, and M. Green. 2005, October. Macanews. AgrimacMacadamias. Issue 7. http://www.agrimac.com.

au/files/agrimac%20news letter%20no.%20 7%20october%202005.pdf.

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

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). S. Afr. J. Bot. 55: 387–393.

Heard, T.A. 1994. Behaviour and pollinator efficiency of stingless bees and honey bees on macadamia flowers. J. Apic. Res. 33: 191–198.

Heard, T.A., and **E.M. Exley.** 1994. Diversity, abundance, and distribution of insect visitors to macadamia flowers. Env. Entomol. 23: 91–100.

Huett, D.O. 2004. Macadamia physiology review: a canopy light response study and literature review. Austr. J. Agric. Res. 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. Agr. Forest Entomol. 14: 81–87.

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. J. Entomol. Soc. S. Afr. 50: 521–523.

(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.

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 Missouri Bot. Garden 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. Ann. Rev. Entomol. 14: 103–124.

Trueman S.J., and **C.G.N. Turnbull.** 1994a. Effects of cross pollination and flower removal on fruit set in macadamia. Ann. Bot. 73: 23–32.

Trueman, S.J., and **C.G. Turnbull.** 1994. Fruit set, abscission and dry matter accumulation on girdled branches of macadamia. Ann. Bot. 74: 667–674.

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

Urata, U. 1954. Pollination requirements of macadamia. Technical Bulletin No. 22, Hawaii Agricultural Experiment Station, University of Hawaii. 40 p.

Wallace, H.M., V. Vithanage, and **E.M. Exley.** 1996. The effects of supplementary pollination on nut set of macadamia (Proteaceae). Ann. Bot. 78: 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. S. Afr. J. Sci. 87: 214–215.