A Low-Cost Trap to Monitor Parasitism of Macadamia Felted Coccid (Hemiptera: Eriococcidae) and Other Scale Insects

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Abstract. We designed and tested a custom-made parasitoid emergence trap that can be installed on macadamia trees in the field to study parasitism of macadamia felted coccid, *Acanthococcus ironsidei* (Williams) (Hemiptera: Eriococcidae). The cost of materials for the trap is approximately $3.00 each, and a trap can be constructed in ~15 min. Optimal methods for determining percent parasitism using these traps are still under development, but two proposed methods gave estimates of 0.24 and 4.85% for mortality due to parasitism by *Encarsia lounsburyi* (Hymenoptera: Aphelinidae). The trap could be an effective and low-cost tool for future parasitism studies or in determining population structure of macadamia felted coccid as it captures parasitoids and other mobile, positively phototactic insects that are present under the covered area of the trap.

Key words: parasitoid, *Macadamia integrifolia*, *Acanthococcus ironsidei*, *Encarsia lounsburyi*

Macadamia felted coccid (MFC), *Acanthococcus ironsidei* (Williams) (Hemiptera: Eriococcidae), is an invasive pest of macadamia nut trees, *Macadamia integrifolia* Maiden & Betche (Proteaceae) in Hawaii (Wright and Conant 2009). MFC was first reported from the South Kona district of Hawaii island in 2005 (Conant et al. 2005) and has since expanded its range to all the macadamia growing areas on the island (Wright and Conant 2009). Adults and young instars (crawlers) of MFC may infest all the above-ground parts of the macadamia tree including leaves, fruits, and racemes, and are commonly observed on the main trunk or lateral branches (Zarders and Wright 2016). MFC inserts its slender stylet into the plant tissue to feed on the sap and causes physiological stress to the tree (Gutierrez-Coarite et al. 2017, Gutierrez-Coarite et al. 2019). Characteristic die-back symptoms with copper-colored foliage become visible on heavily infested trees. Growers rely on horticultural oils and insect growth regulators to manage MFC in the field (Ironside 1978, Gutierrez-Coarite et al. 2019). An imported biocontrol agent, *Metaphycus macadamiae* Polaszek & Noyes (Hymenoptera: Encyrtidae), is currently in quarantine in Hawaii (Polaszek et al. 2020).

MFC is native to Australia and populations in macadamia there are often kept in check by natural enemies, particularly by coccinellid beetles and two species of parasitoids (*Encarsia* sp. and *Metaphycus* sp.) (Ironside 1978). The absence of predators and parasitoids in places where MFC has been introduced (Hawaii and South Africa) allows populations to grow to damaging levels if left uncontrolled. In Hawaii, pest outbreaks and serious damage to macadamia trees in commercial

orchards havr been reported since its introduction (Wright and Conant 2009). Initial surveys for natural enemies in Hawaii indicated that several species of coccinellids feed on MFC, but their ability to suppress populations has not been quantified (Conant and Hirayama 2005, Gutierrez-Coarite et al. 2019). A local resident parasitoid *Encarsia lounsburyi* (Berlese & Paoli) (Hymenoptera: Aphelinidae) has been reported to parasitize MFC in Hawaii (Conant and Hirayama 2005). Surveys using sticky cards have confirmed the presence of *E. lounsburyi* in the major macadamia growing areas of Hawaii Island (Gutierrez-Coartite et al. 2017a). Gutierrez et al. (2017b) estimated parasitism of MFC by counting exit holes present on infested branches collected from the field (Gutierrez-Coartite et al. 2017b). *E. lounsburyi* is a globally distributed aphelinid parasitoid that has been reported to be associated with over 50 armored scale host species (Noyes 2016) and is morphologically similar to *Encarsia citrina* Craw (formerly *Aspidiotiphagus citrinus* subsp. *citrinus* Craw), a known parasitoid of MFC in Australia (Ironside 1978). Much of the biology of *E. lounsburyi* remains to be explored. It has been reported parasitizing 2nd instar to adult stages in armored scales (Làzaro-Castellanos et al. 2021). Aphelinidae parasitoids are generally koinobiont endoparasitoids, and adult wasps generally emerge from 3rd instar to adults in armored scales (Stocks and Evans 2017).

Determining the parasitism rate is important in assessing the impact and ecosystem services provided by biological control agents (Losey and Vaughan 2006). Parasitism rate can be determined by rearing field-collected hosts in the laboratory for emergence of parasitoids, or for endoparasitoids, by microscopic dissection of host specimens (Sow et al. 2019). These methods are labor-intensive and often not scalable for estimating parasitism across many field sites (Sow et al. 2019). Rearing the insect host in the laboratory under artificial conditions may also affect parasitoid survival and emergence rate (Sow et al. 2019). Molecular tools can also be used to examine parasitism and host-parasitoid interactions (de Leon et al. 2010, Kitson et al. 2019); however, in scenarios where there is limited parasitism, as in the case of MFC in Hawaii, molecular approaches may be prohibitively costly. Traditional methods of capturing parasitoids such as use of a Malaise trap or sticky cards are non-specific and capture non-target species (Fraser et al. 2008, Holthouse et al. 2021).

MFC population densities are typically characterized by counting crawlers or adults from a unit area of the bark (Guitierrez-Coarite et al. 2017). Developing a comparable method to estimate parasitism rate in the field by collecting parasitoids from naturally occurring MFC on the tree bark would be ideal for future parasitoid establishment studies. Nalepa (1987) developed an inexpensive emergence trap for monitoring scale parasitoids in the field using plastic photographic film canisters. Film canisters are no longer readily available, but a similar trap design might be useful in evaluating macadamia felted coccid parasitoids and parasitism. The objectives of this study were to 1) to design and test a low-cost parasitoid emergence trap, and 2) to estimate parasitism rate of MFC in Hawaii macadamia orchards using the emergence trap.

Materials and Methods

Design and construction of the parasitoid emergence trap. The design concept for the parasitoid emergence trap was adopted from a study conducted to monitor parasitoids in white peach scale, *Pseudaulacaspis pentagona* (Targioni) (Nalepa 1987) on peach *Prunus persica* (L.) Batsch. An opaque black PET bullet

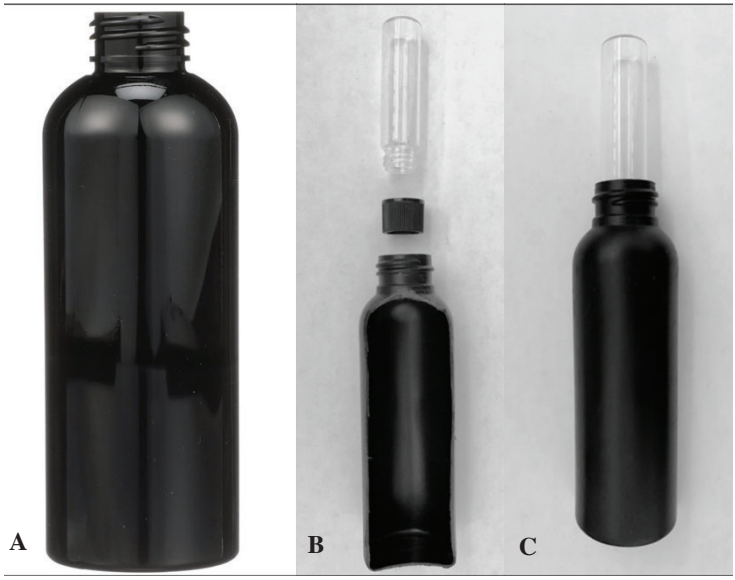

Figure 1. A. A low-cost 100 ml PET bottle can be used to construct a parasitoid emergence trap to study parasitism of MFC in the field. B. Basic components of the parasitoid emergence trap showing the PET bottle with modifications allowing for attachment to the tree branch and insertion of a glass collection vial. C. A fully assembled trap.

round bottle (100 ml, M & H Plastics, Winchester, VA) (Fig.1 A) was used to construct the emergence trap. One side of the bottle, starting from the base of the neck, was cut and removed to create an enclosure that could be attached to the branch (Fig. 1B). A broad hole was cut into the cap of a 10 ml insect collection vial and the cap was then inserted into the neck of the PET bottle and secured in place with hot glue. This design allows easy attachment and removal of the insect collection vial from the PET bottle while the trap is attached to the tree (Fig. 2). Emerging wasps and other insects exhibiting positive phototaxis might move from the dark interior of the bottle toward the light in the collection vial. The open area of the trap had a width of 3.5 cm and a height of ~10 cm; thus, each trap covered approximately 35 cm^2 (5.4 sq. inch) of bark when installed on the trees. The trap can be attached to

Figure 2. The parasitoid emergence trap installed on a macadamia tree branch infested with macadamia felted coccid.

the tree branch using a zip-tie, with its cut-open side covering the MFC infestation on the branches and the sides can be sealed using reusable clay (Sargent Art, Mexico). The cost of materials for the trap is approximately US$ 3.00 each, and a trap can be constructed in about 15 min.

Field testing of the emergence trap. A study was initiated in October–November 2020 in two macadamia fields to test the trap. The first field was located in South Kona (19°08'03.9"N 155°50'37.8"W) and the second field was located in Honokaa (20°05'11.3"N 155°31'12.8"W). A third field was selected in Pahala (19°14'08.2"N 155°27'35.5"W) in 2020 but not sampled until 2021 because of low MFC numbers at this location. At each location, infested macadamia trees were identified by the presence of white felted sacs (the felted covering of adult MFC). The presence of active populations of MFC was confirmed by examining the tree branches or main trunk for the mobile immature stages using a handheld microscope but without quantifying MFC numbers. Once active populations were identified, the trap was placed over an area of the branch where MFCs were concentrated. The trap was attached to the tree using zip-ties and the gaps between the sides of the PET bottle and branch were sealed using the reusable modeling clay (Fig. 2). Three trees were selected from each field and three traps were placed in each tree on different branches or different areas of the same branch where MFC was present. After one week, the glass vial with collected insects was removed from each trap, filled with 75% ethanol, and labelled for later examination. The traps were then moved to a new position on the same tree with a new vial. This process was repeated for three weeks for each tree for a total of nine collections per tree. The contents of the vials were examined for parasitoids and other insects.

Parasitism rate study. A second trial was conducted in October–November 2021 to determine weekly parasitism rate by collecting emerging parasitoids and estimating the number of MFC adults under the traps. Parasitoid traps were installed in three blocks of macadamia trees in South Kona where a higher number of parasitoids were captured during the preliminary testing. Three traps were installed on each of 6–9 trees and samples were collected weekly for a period of 3–5 weeks from each tree. The density of MFC was determined by taking close-up photographs at a fixed distance using a digital microscope (Dino-Lite, Torrance, USA) at ~70x magnification from areas of bark with comparable pest density immediately adjacent to each trap. The photographs were taken after the installation of the traps either on the same day or next day to accurately reflect the population levels at the time of installation. Each photograph covered an area of 1.08 cm^2 on the bark and six photographs were collected from areas next to the traps every week and averaged to estimate MFC density (mean number/cm^2) in the emergence traps.

Parasitoid behavior. A video recording of *E. lounsburyi* parasitizing MFC adults in the field was made using a handheld digital microscope camera (Dino-Lite, Torrance, USA) at ~70x and 140x magnification. The video was recorded by gradually moving the camera while on 'record' mode over MFC-infested areas and stopping when a *E. lounsburyi* wasp was spotted. The video was used to describe general behavior of the parasitoid including host searching and antennation of the host, and the approximate time taken to complete the process of parasitism.

Results and Discussion

Field testing of the emergence trap. Over a period of three weeks, 92 adult parasitoids were captured in nine traps at

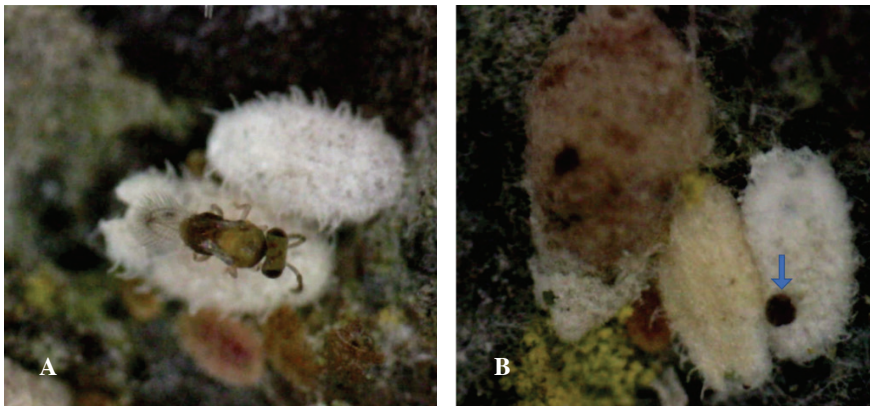

Figure 3. A. *Encarsia lounsburyi* parasitizing an MFC adult. B. A circular parasitoid emergence hole on an adult male MFC host, indicating parasitism.

Table 1. Parasitoids and other insects captured from traps placed over macadamia felted coccid-infested branches for a three-week period.

Site	No. traps	No. samples	*Encarsia lounsburyi*	Bycatch
South Kona	9	27	92	MFC, Psocoptera
Honokaa	9	27	4	MFC
Pahala	9	27	0	MFC, Psocoptera, Curculionidae

South Kona (Table 1). All of the parasitoids were of the same species. Representative specimens were sent to Dr. Gregory Evans, Systematic Entomology Laboratory, USDA-APHIS PPQ, 10300 Baltimore Avenue, Beltsville, Maryland, and identified as *Encarsia lounsburyi* (Fig. 3). The traps also captured 24 MFC adult males and crawlers and two Psocoptera adults (species not identified) (Table 1).

Four *E. lounsburyi* wasps were captured from nine traps over a period of three weeks from Honokaa. A total of eight MFC males and crawlers were also captured in vials during the sampling period. No other species was found in the traps. No parasitoids were found from trapping in Pahala, but a total of 16 MFC males and crawlers, five Psocoptera adults, and four adult ambrosia beetles (Curculionidae: Scolytinae) were found in the traps. Installation of the trap on the trees required about 15 minutes per trap including the time required to seal the base of the trap using reusable clay. The parasitoids were generally stuck to the condensation along the inner walls of the glass vials close to the neck suggesting that they were trapped soon after emergence.

Parasitism rate study. A total of 36 *E. lounsburyi* wasps were collected from the three blocks of macadamia during a five-week study period, with a mean of 0.32 ± 0.08 parasitoids per trap per week (Table 2). The mean number of adult MFC (males and females) under the traps was estimated at 643.31 ± 46, which with a trap area of 35 cm^2 (5.4 sq.in), gives an estimated average density of 18.4 MFC per cm^2. The weekly mean percent

Table 2. *Encarsia lounsburyi* emergence from macadamia felted coccid-infested branches in South Kona.

Block	Tree	Obs.	Total no. *E. lounsburyi*	Mean no. *E. lounsburyi* ± SEM	Mean no. MFC emerged ± SEM	Mean MFC estimated (35 cm^2)	Rate of estimated parasitism (%)
1	1	15	2	0.13 ± 0.13	12.00 ± 2.38	781 ± 177	0.02
	2	15	4	0.33 ± 0.19	4.50 ± 2.32	494 ± 102	0.07
	3	15	0	0.00 ± 0.00	2.58 ± 1.01	679 ± 205	0.00
2	4	15	17	1.13 ± 0.55	8.87 ± 3.22	429 ± 18	0.26
	5	15	5	0.33 ± 0.16	4.20 ± 1.65	781 ± 123	0.04
	6	15	2	0.13 ± 0.13	3.33 ± 0.98	426 ± 43	0.03
3	7	12	4	0.33 ± 0.19	6.92 ± 1.77	674 ± 153	0.05
	8	9	1	0.11 ± 0.11	8.00 ± 2.61	796 ± 158	0.01
	9	9	1	0.11 ± 0.11	5.44 ± 1.76	862 ± 76	0.01
Average				0.32 ± 0.09	6.27 ± 0.74	643 ± 46	0.06

parasitism rate based on the total population present under the traps was estimated as 0.06% (0.24% monthly). Gutierrez-coartite et al (2017b) estimated parasitism in the range of 1.3% (control plots) to 4.3% (modified understory plots) by estimating parasitoid exit holes on MFC on infested branches. While the methods differ, both approaches generally agree on the presence of low levels of baseline parasitism. In our study, at least one *E. lounsburyi* was collected from eight out of the nine trees sampled; however 17 out of the 36 *E. lounsburyi* were collected from a single tree. *E. lounsburyi* was collected from two trees during the first week of sampling and increased to six out of nine trees during the final week of sampling.

Parasitoid behavior. Two separate instances of parasitism were recorded in the field. In the first instance, *E. lounsburyi* was observed parasitizing an adult female MFC. After landing on the female, the parasitoid was observed continuously walking over the host while tapping on the host body using the antennae for ~30 seconds before bending the abdomen for presumptive insertion of the ovipositor. The oviposition period was approximately 60 seconds before the wasp moved off the host. In the second instance, *E. lounsburyi* was observed parasitizing two male MFC adults within three minutes. For each host, the parasitoid spent 30–50 seconds walking over the host while tapping antennae before stopping and bending the abdomen for the insertion of the ovipositor. A video of *E. lounsburyi* parasitizing an MFC adult is posted at this

link: https://youtube/t3CC-1B6Yoc.

Estimating parasitism rate in MFC. We designed and tested a custom-made parasitoid emergence trap that can be installed on macadamia trees in the field to study parasitism of macadamia felted coccid. In the preliminary testing, a resident aphelinid parasitoid *E. lounsburyi* was captured in the trap from two locations. In the second study conducted in South Kona, the parasitoid was captured from eight out of nine trees sampled. We estimated a weekly percent parasitism rate by collecting the *E. lounsburyi* using the emergence trap and estimating MFC adults under the traps using close-up photographs. The trap efficacy was not validated by holding a known number of samples (parasitoids) and quantifying the emergence; however, inside of the traps were rinsed with alcohol to account for any parasitoids or hosts that might have stuck to the inside of the traps and added to the collecting vials during each sample collection. It might be ideal to determine the efficacy of the trap using a known number of parasitoids as the microenvironment inside the traps might affect the emergence of the parasitoids.

It is unclear what impact an average weekly parasitism rate of 0.06% by *E. lounsburyi* may have towards MFC control. The mean developmental time of MFC from egg-hatching to final instar is about 13–19 days for males and 29–36 days for females (Zarders and Wright 2016). Assuming a mean developmental time of 30 days until crawlers become sedentary—a likely target stage of parasitism—a mean weekly percent parasitism of 0.06 might account for a generational parasitism rate of 0.24%. Alternatively, the parasitism rate could be estimated from the number of MFC and *E. lounsburyi* captured in the emergence trap. Because our emergence trap captures both MFC (adults males and crawlers) and parasitoids, parasitism rate could be estimated indirectly (apparent parasitism) using the equation:

$$\frac{\text{Parasitoids emerged}}{\text{MFC emerged + parasitoids emerged}} \times 100$$

(McAuslane et al. 1993, Qureshi et al. 2009)

provided the age of the MFC cohort is known. Using this equation with the current data, we estimated the parasitism rate as 4.85% on a cohort that was mostly male adults. However, this estimate needs further refinement as the trap does not capture females and very few crawlers.

These two estimates of parasitism rate (0.24% and 4.85%) present two potential ways to use these types of data for further research. Another approach to precisely estimate parasitism rate involves removal of the existing MFC population and waiting for a fresh cohort of MFC to repopulate the cleared area. Estimating parasitism on a fresh cohort might be more reliable because determining the approximate age and population structure (the proportion of males, females, and crawlers) of the new cohort is relatively easy. However, this might take several weeks and may be practical only when the infestation level is high and in trees not treated with insecticides. A comparable study was conducted on *Encarsia citrina* Craw, a biocontrol agent of the elongate hemlock scale *Fiorinia externa* Ferris (Hemiptera: Diaspididae) (Abell and Van Driesche 2011). *E. citrina* parasitism was determined by excluding or including the parasitoids on fresh cohorts of scales in the field. Similar exclusion studies could be conducted with MFC and *E. lounsburyi*. Determination of any preference by the parasitoid for female or male hosts could also be important in future studies.

Further studies in the lab and field are needed to understand the generational mortality caused by *E. lounsburyi* and other host-parasitoid interactions. The

density of MFC was estimated based on the intact host life stages visible (male and female adults) in close-up photographs. Determining the phenology of MFC in field populations is a difficult task because of the continuous overlapping generations and difficulties determining if males have emerged from their felted sacs. We also observed a non-uniform distribution of *E. lounsburyi* in the orchard. Up to 47% of all *E. lounsburyi* collected in the second study was from a single tree. Parasitism appeared to increase as sampling progressed from early October to Mid-November 2021 as suggested by a greater number of trees with at least one parasitoid per trap. Therefore, seasonality in parasitism may be important. Variability in the number of parasitoids collected over different weeks was not tested statistically because of low sample size.

A previous study of the biology of MFC in macadamia orchards reported finding *E. lounsburyi* in South Kona, Honokaa and Pahala (Gutierrez-Coartite et al. 2017). Though the numbers from that study are not readily comparable because of the differences in the sampling method, both studies agree on the presence of the parasitoid in South Kona and Honokaa. Gutierrez-Coartite et al. (2017b) reported increased presence of *E. lounsburyi* in macadamia plots planted with wildflowers. Future studies should investigate the use of habitat modification to enhance conservation biological control of MFC.

Future biological control releases. Assessing parasitoid establishment in a new habitat and calculation of rate of parasitism in the field are critical in assessing the success of biological control programs. The trap described in this paper is effective in capturing parasitoids and other mobile positively phototactic insects that are present under the covered area of the trap and will be a low-cost option for future parasitoid studies. Easy installation and maintenance requirements make this emergence trap suitable for continuously monitoring seasonal abundance and fluctuations of any parasitoid species in relation to the density of MFC or other scale insects. The trap may be useful in the near future with the forthcoming release of the MFC-specialist parasitoid *Metaphycus macadamiae* in Hawaii.

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