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ORIGINAL RESEARCH ARTICLE

Feeding habits, movement, and reproduction of the predatory flat bark beetles *Cathartus quadricollis* (Coleoptera: Silvanidae) and *Leptophloeus* sp. (Coleoptera: Laemophloeidae) in Hawaii coffee and macadamia nut

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Abstract
Hawaii has a multitude of scolytine (Coleoptera: Curculionidae: Scolitinae) pests including *Hypothenemus hampei*, coffee berry borer, which is apest of coffee, *Hypothenemus obscurus,* tropical nut borer, a significant pest of macadamia nut, and *Xylosandrus compactus*, black twig borer, a pest of many tropical and ornamental crops. The flat bark beetles, *Carthartus quadricollis* (Coleoptera: Silvanidae) and *Leptophloeus* sp. (Coleoptera; Laemophloeidae), are known to predate on coffee berry borer and tropical nut borer but their natural history (feeding habits, reproduction and movement) are poorly understood. Studies were conducted using molecular, field, and laboratory assays to examine 1) flat bark beetle reproduction and movement in coffee and the broader agricultural landscape, 2) establishment of augmentative releases for biological control, and 3) predation rates on *H. hampei*, *H. obscurus* and *X. compactus*. Various life stages of *C. quadricollis* and *Leptophloeus* sp. were found in seven different plant species common to the agricultural landscape around coffee farms, suggesting these predators are feeding and reproducing on theses hosts. Molecular analysis indicated that *C. quadricollis* and *Leptophloeus* sp. predated on *H. hampei, H. obscurus* and *X. compactus* in coffee, macadamia nut, and mixed coffee-macadamia nut farms. Laboratory reared predators were recaptured near release sites on coffee farms at 1, 2, and 7 weeks after augmentative releases. Predation of *C. quadricollis* on *H. hampei* eggs placed inside artificial coffee berries in coffee farms was about 40%. Predators *C. quadricollis* and *Leptophloeus* sp. are natural enemies of some significant agricultural scolytine pests in Hawaii and have excellent potential for biological control with augmentative releases.

Keywords *Coffea* · *Macadamia* · Biological control · Predators · *Hypothenemus hampei* · *Hypothenemus obscurus* · *Xylosandrus compactus* · *Cathartus quadricollis* · *Leptophloeus sp*

Introduction

Among the most economically important of scolytine pests in Hawaii are the beetles coffee berry borer (*Hypothenemus hampei* (Ferrari)), tropical nut borer (*Hypothenemus obscurus* (F.)), and black twig borer (*Xylosandrus compactus* Eichoff) (Coleoptera: Curculionidae: Scolytinae). *Hypothenemus hampei*, a recent invasive species discovered in 2010 in Hawaii, bores into the blossom end of ripening coffee (*Coffea arabica* L.) and if left unmanaged, can damage >90% of the crop (Follett et al. 2016). *Hypothenemus obscurus* causes significant damage to macadamia nut (*Macadamia integrifolia* F. Muell) kernels, especially if harvests are infrequent and the nuts are left undisturbed post-abscission (Jones 2002). *Xylosandrus compactus* attacks >200 host plants, including coffee and macadamia nut, as well as other tropical crops such as cacao (*Theobroma cacao* L.), avocado (*Persea americana* Mill), and a variety of nursery, ornamental and native Hawaiian plants (Greco and Wright

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2015). Both *H. obscurus* and *X. compactus* are found on all major islands in the state; *H. hampei* was initially detected on the island of Hawaii where much of the state's coffee is grown and has since spread to other islands (Messing 2017).

Management of these scolytine pests varies depending on the species, but due to their cryptic nature, requires a multifaceted approach using a variety of methods. In Hawaii, *H. hampei* and *H. obscurus* management is centered on timely, frequent harvests and extensive field sanitation practices. The fungal entomopathogen, *Beauveria bassiana*, has been useful for coffee farmers against *H. hampei* when the sprays are well-timed based on the individual infestation dynamics of the farm. Management of *H. obscurus* in macadamia nut focuses on frequent harvests. For *X. compactus*, the only recommended practice is removing and destroying infected branches (Greco and Wright 2012). Pesticide treatments against scolytine pests are often cost-prohibitive as they are labor-intensive, expensive, and do not penetrate into the plant tissue where the beetles occur, there by greatly reducing their efficacy (Kawabata et al. 2017).

Natural predators have the potential to help reduce pest populations in conjunction with other management practices. The silvanid flat bark beetle, *Cathartus quadricollis* (Guérin-Méneville) (Coleoptera: Silvanidae), and the lined flat bark beetle, *Leptophloeus* sp. (Coleoptera: Laemophloeidae), are two common beetles found in the agricultural landscapes in Hawaii (Jones 2002; Follett et al. 2016). Previous research in coffee and macadamia nut has shown their utility as predators against *H. hampei* (Sim et al. 2016; Follett et al. 2016) and *H. obscurus* (Jones 2002). Laboratory feeding assays demonstrated the capacity for adult and larval *C. quadricollis* and *Leptophloeus* sp. to feed on all *H. hampei* life stages. These predators are widely distributed in the coffee growing areas on the island of Hawaii, but feed mainly in overripe and dried coffee on the tree rather than coffee on the ground or in ripening berries where initial crop damage occurs (Follett et al. 2016). Berlese funnel extraction of *C. quadricollis* and *Leptophloeus* sp. from dried coffee bean on the tree indicated that predator numbers can be relatively high, with as many as 23 adult predators per 150 whole fruit sample. *C. quadricollis* was also found not susceptible to infection by the fungal biopesticide *Beauveria bassiana* which is used for field control of *H. hampei* on coffee. *C. quadricollis* and *Leptophloeus* sp. can be propagated readily on a diet of cracked corn and cornmeal, and *C. quadricollis* is currently being raised and released for augmentation biological control of coffee berry borer (Follett et al. 2016).

Research was conducted to better understand the ecology of *C. quadricollis* and *Leptophloeus* sp., particularly as augmentative biological control agents of *H. hampei,* and also as predators of *X. compactus* and *H. obscurus* in macadamia nut and other plants in the coffee landscape. Due to their relatively small size and the cryptic feeding habits of their scolytine hosts, reproduction, diet breadth, rates of predation, and movement have been difficult to study in flat bark beetles (Thomas 1993; Thomas 2002). The fruiting structures of several landscape plants near coffee were sampled to identify where predators are reproducing. Molecular markers were developed for *H. obscurus* and *X. compactus* in addition to those developed for *H. hampei* (Sim et al. 2016) and were used to study predation and movement by *C. quadricollis* and *Leptophloeus* sp. in coffee and non-coffee habitats. Field cage and artificial coffee berry experiments were conducted to determine rates of predation. Further, predator release and recovery studies were performed to examine movement of predators post-augmentation and determine the efficiency of mass releases. These studies are a companion to earlier studies with *C. quadricollis* and *Leptophloeus* sp. (Follett et al. 2016) and will expand knowledge about the ecology of these two poorly studied families of flat bark beetle predators.

Materials and methods

Wild and colony insects

Both wild and colony insects were used for the experiments. Wild adult *C. quadricollis* and *Leptophloeus* sp. were collected from coffee raisins (dried coffee on the tree), macadamia nut sticktights (old nuts that have not abscised from the tree), and leguminous plant seed pods in the field and on farms in South Kona and Pahala regions of Hawaii Island from April 2015 – February 2017 and used in studies on molecular detection of prey and determining reproductive plant hosts. For mass release experiments, *C. quadricollis* colonies were reared in the laboratory in 2 L plastic buckets (Hi-Plas, Mira Loma, CA) with screened lids on a standard 4:1 cracked corn:cornmeal dry diet (Follett et al. 2016) in an insect growth chamber (Darwin Chambers, St. Louis, MO) set to 28 °C and 70% RH. Scolytid prey were not reared on a diet and were collected and used live from the field. *Xylosandrus compactus* adults were collected from coffee laterals and verticals; *H. obscurus* adults were collected from macadamia nut sticktights; and *H. hampei* adults were collected from infested coffee berries.

Reproductive host plants

Although *C. quadricollis* and *Leptophloeus sp.* adults had been found in coffee raisins, no larvae or other life stages were previously detected during coffee raisin extractions (Follett et al. 2016). Therefore, a study was conducted to identify reproductive plant hosts. A thorough sampling of common plants found in the agricultural landscape around the coffee growing region was conducted April 2015 – February 2017 to determine where reproduction was occurring. Plant tissue was

collected and processed either via modified Berlese funnel or manual dissection and observations of *C. quadricollis* and *Leptophloeus* sp. life stages were recorded (Table 1).

Reproduction in coffee

It was previously believed that coffee raisins were not suitable for *C. quadricollis* nor *Leptophloeus* sp. reproduction (Follett et al. 2016), but this assessment was made from Berlese extraction of coffee berry samples rather than by manual dissection and inspection, which is more precise. A total of 2500 raisins were collected from five farms ($N = 500$ raisins per farm) in South Kona in December 2015. A subset of 250 raisins ($N = 50$ per farm) were collected and manually dissected. The remaining 2250 raisins ($N = 450$ per farm) were extracted using a modified Berlese funnel with a 40 W bulb for 48 h. Seventy-two larvae were recovered from both dissected and berlese funnel extracted raisins and each individual was placed in its own 30 g container with 1 g of 4:1 ratio cracked corn/cornmeal mixture, which is the standard rearing diet for both *C. quadricollis* and *Leptophloeus* sp. (Follett et al. 2016). The 72 containers with larvae and diet were placed in an insect growth chamber (Darwin Chambers) and left to develop at 28 °C and 70% RH. After 2 weeks, containers were evaluated to identify adult beetles that had successfully eclosed.

Release and recapture of *Cathartus quadricollis*

Coffee farmers are raising and releasing *C. quadricollis* adults, but nothing was known about their movement after release. A study was conducted to determine if *C. quadricollis* could be recovered from coffee berries infested with *H. hampei* near release sites and used as an indicator of movement. Three unmanaged coffee farm sites were found to have high infestations of *H. hampei*, an established population of wild *Leptophloeus* sp., but no *C. quadricollis*. These sites were selected to conduct mass releases. *C. quadricollis* were mass-reared in the laboratory approximately 4 months before release in preparation for the experiment. Releases were conducted at one site in Holualoa, HI in February 2015 and two sites in Pahala, HI in February 2016, when raisins were abundant on the coffee trees. An experimental plot of 15 m × 15 m at each site was identified for mass release of approximately 10,000 *C. quadricollis* adults. Adults were released with their corn diet in equal amounts at the crotch of each coffee tree in the experimental plot, which is the recommended method for farmers to distribute beetles (see UH CTAHR extension video at https://www.hawaiicoffeeed.com/predators-of-cbb.html). Coffee raisins were sampled on the branches at one and 2 weeks prior to the mass release, and at 1, 2, and 7 weeks post-release. Ten 4 oz. Whirl-Pak bags (Zefon International, Ocala, FL) of *H. hampei*-infested raisins were collected on each sampling date per site. Raisins were placed in a modified Berlese funnel and extracted for 48 h to determine the number of flat bark beetles present.

Molecular detection of prey

Collection of arthropods Two types of collections of target insects were collected from the wild for molecular detection assays – those from plant reproductive structures (e.g. beans, nuts, pods) scattered across the landscape on different farms and other areas, and from plant reproductive structures in four types of agricultural habitats common to the landscape: monotypic coffee farms, monotypic macadamia nut farms, mixed coffee/macadamia nut farms, and mixed vegetation near coffee farms. Wild beetles were collected from coffee raisins, macadamia nut sticktights, and seed pods of potential alternate host plants. All beetles were identified using a Leica Wild M3Z stereomicroscope. Laboratory controls for *C. quadricollis* and *Leptophloeus* sp. were collected from colony beetles reared on a cracked corn/cornmeal diet only (no feeding on prey). Wild and colony beetles were individually

Table 1 Host plants, life stages found (°Family Rubiaceae, drupes sampled; ^Family Proteaceae, sticktight nuts sampled; *Family Fabaceae, seed pods sampled)

Plant	common name	Leptophloeus sp.				Cathartus quadricollis			
		egg	larvae	pupae	adult	egg	larvae	pupae	adult
Coffea arabica°	coffee		X		X	X	X	X	X
Macadamia integrifolia^	macadamia nut		X	X	X	X	X	X	X
*Leucaena leucocephala**	koa haole		X	X	X		X	X	X
*Samanea saman**	monkeypod		X		X		X		X
*Inga spp.**	ice cream bean		X		X		X		X
*Senna spp.**	scrambled egg plant				X				
*Acacia koa**	koa				X				
*Erythrina variegata**	coral tree		X		X				

placed in a labeled 1.5 mL microcentrifuge tube filled with 95% ethanol. Tubes were stored in the −80 °C freezer until DNA extraction. Each sample of predator genomic DNA underwent PCR using the three species-specific scolytid primers and a positive predator control to determine predation, and products (sample results) were visualized on a 2% agarose gel.

DNA extraction and sequencing DNA extraction was performed using a NucleoMag Tissue kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) following the manufacturer's standard protocol. Individual beetles were rinsed in sterile deionized water, dried, and then placed in 2 mL screw cap tubes with T1 buffer, proteinase K, and 0.1 mL of 1 mm sterile zirconium silicate beads (BPI Tech, San Diego, CA). Samples were homogenized using a FastPrep-24 homogenizer (MP Biomedicals, Santa Ana, CA) at a speed of 4.0 m/s for 40 s. After overnight incubation at 56 °C, samples were extracted on a Kingfisher Flex Purification System (Thermo Fisher Scientific, Waltham, MA). The resulting DNA was eluted into 100 μL of NucleoMag Tissue MB6 buffer. To verify quantity and quality, genomic DNA was separated on a 1% agarose gel alongside an exACTGene 1 kb Plus DNA Ladder (Thermo Fisher Scientific), and a Qubit 2.0 fluorometer (Thermo Fisher Scientific) assay was performed using the dsDNA High Sensitivity Kit according to the manufacturer's standard protocol.

Polymerase chain reaction (PCR) was performed to amplify a 658-bp fragment from the COI region using the primers Lep-F1 5′ – ATTCAACCAATCATAAAGATATTGG – 3′ and Lep-R1 5′ – TAAACTTCTGGATGTCCAAA AAATCA – 3′, which is highly conserved in many invertebrate taxa and optimized for Lepidoptera, but has worked well for many species in Coleoptera (Hebert et al. 2004). Each PCR totaled 25 μL containing: 12.5 μL of One*Taq* Quick-Load 2X Master Mix with Standard Buffer (New England Biolabs, Ipswich, MA), 0.5 μL of forward primer, 0.5 μL of reverse primer, 10.5 μL of nuclease-free water, and 1.0 μL of genomic template DNA. Amplification was performed in a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories, Hercules, CA) under the following cycling conditions: initial denaturation at 94 °C for 1 min; 35 cycles of denaturation at 94 °C for 30 s; annealing at 55 °C for 30 s; an extension at 68 °C for 30 s; and a final extension at 68 °C for 5 min. PCR products were then separated on a 2% agarose gel with Midori Green Advance DNA Stain (Nippon Genetics Europe GmbH, Düren, Germany), alongside an exACTGene 100 bp PCR DNA Ladder (Thermo Fisher Scientific). The gel was run at 90 V for 45 min and visualized with a Bio-Rad Gel Doc XR+ Imaging System (Bio-Rad Laboratories).

Primer design and specificity After verifying the quality of the amplicons using gel electrophoresis, the PCR products were purified using Agencourt AMPure XP (Beckman Coulter, Brea, CA) on a Kingfisher Flex System using their standard protocol. The cleaned PCR products were sent to a sequencing service company (Eurofins Genomics, Louisville, KY). The resulting sequences were aligned using Sequencher 5.0 analysis software (Gene Codes Corporation, Ann Arbor, MI) and analyzed to locate ideal regions for creating scolytid-specific primers. Primers for *C. quadricollis* and *Leptophloeus* sp. were previously designed by Sim et al. (2016).

The scolytid-specific primers were designed by locating regions that were common within species, but different amongst species (Table 2). All primers were tested extensively using gradient PCR to determine an appropriate annealing temperature that would yield specific amplification and no contamination nor non-specific banding. It was determined that all primers could be amplified using one annealing temperature of 59 °C. A 10-fold serial dilution was conducted to determine primer sensitivity of prey DNA (Fig. 1).

Predation rates in sleeve cages and artificial berries

Sleeve cage study A field cage study was conducted to determine if *C. quadricollis* augmentation results in increased predation on *H. hampei* life stages. This experiment was conducted at three coffee farms in South Kona, HI. Coffee branches were selected for caging based on similarity in the numbers of berries per branch and the percentage of berries infested by *H. hampei* (by observing the presence (infested) or absence (uninfested) of a boring hole at the blossom-end of the berry). Once selected, 16 (four replicates of four treatments) coffee branches per farm were enclosed in 70 × 30 cm white mesh insect rearing sleeves (BugDorm, Taiwan) and sealed by tying off the ends. A custom internal skeleton made of hooped wire was inserted over the branch before the sleeve was put on to improve air flow and prevent collapse during rainfall. The sleeves were enclosed around ripening coffee, as flat bark beetles do not normally inhabit this

Table 2 Species-specific primers for three scolytine pest species

Species	Forward primer (5′ ➔ 3′)	Reverse primer (5′ ➔ 3′)	Product size (bp)
Hypothenemus hampei	TACAATTGTTACTGCACACGC	TGGCAAGGTCTACGGAAGA	236
Xylosandrus compactus	TCCGAACAGAACTTGGAACT	GGTTAATCTAGGTGGAAGAAGCC	179
Hypothenemus obscurus	CGGAAACTGATTAGTACCAC	GGTAGAAATGAAGGATTGCC	227

Fig. 1 Serial dilutions with starting concentrations of 2 ng/μL. DNA can be detected using species-specific primers created for each species. There is variation in the sensitivity of detection for each species

stage (Follett et al. 2016). These coffee berries were left to mature inside sleeves until the raisin stage, where treatments were then added. Two controls were established – a baseline control to serve as a metric for an initial infestation level at the beginning of the experiment (control at time 0), and a *H. hampei* only control, where sleeves were left unaugmented to determine infestation level at the end of the experiment. The two treatments were the addition of 10 *C. quadricollis* adults and 40 *C. quadricollis* adults. After 3 weeks, the branches were clipped and collected in 1 gal. labeled zip-top plastic bags and placed in a cooler. Raisins were manually dissected and processed within 72 h of removal from the field. All immature *H. hampei* life stages and numbers of *C. quadricollis* were quantified per bean. For each farm, the average baseline value for each life stage was calculated from the baseline control branches, and the values for the *H. hampei* only, 10 *C. quadricollis* and 40 *C. quadricollis* were subtracted from the baseline control to estimate change over time. When the final number for a life stage in a treatment was less than the baseline control value, this indicated a decrease in the number of individuals in that life stage. This provided a data set of final values which represented change over time for each treatment; therefore, if an end value was less than the baseline the values were negative and represented a decrease in life stage from the baseline. Those values were then log-transformed and subjected to ANOVAs and post-hoc Tukey's HSD tests using R statistical software v.3.3.3.

Artificial coffee berries Quantifying predation by predators inside coffee berries is difficult because the number of *H. hampei* life stages present at any time point is unknown and predation cannot be observed inside the coffee berry where *H. hampei* reside. Artificial coffee berries were constructed, allowing the insertion of a known number of *H. hampei* before placement in the field. These artificial berries were constructed from a 16 mm diameter wooden bead. The bead was sawed in half and hollowed out using a router to create a 10 mm diameter chamber inside. A hole was drilled into the side of the bead using a 1 mm drill bit. The 1 mm size of the hole was comparable to that created by an adult *H. hampei* while boring into a coffee berry. When the two halves of the hollow bead were joined, using masking tape along the seam, the only entry into the bead was through the *H. hampei*-sized hole, thus simulating the structure of an infested coffee berry.

To estimate the rate of predation, 20 fresh *H. hampei* eggs were placed inside the chamber of the artificial berry and sealed. Four artificial berries were attached to a 1-m bamboo stake, which was attached horizontally to a 1.5 m fiberglass plant stake inserted in the ground within in a row of coffee trees (Fig. 2). Tanglefoot was applied to the base of the plant stake to prevent access to the artificial berries by ants. At each of six coffee farms, three bamboo poles with four artificial berries per pole, were staked at three different locations (12 artificial berries per farm on each date) on 3 different dates between March and August 2017. Forty-eight hours after placement, the artificial berries were removed from the field and brought back to the laboratory where remaining eggs were counted.

Results

Reproductive host plants

C. quadricollis and *Leptophloeus* sp. were associated with a variety of plants in the agricultural landscape including coffee. Various life stages (eggs, larvae, pupae, and adults) of the two species were collected from many of the host plants sampled, suggesting flat bark beetle reproduction (Table 1) in the reproductive structures of coffee, macadamia nut, *Leucaena* sp., *Samanea* sp., *Inga* spp., and *Erynthrina* sp. Of the 72 larval predators collected from coffee raisins, 56 (78%) were successfully reared to adulthood, with a composition of 43 *Leptophloeus* sp. (77%) and 13 *C. quadricollis* (23%).

Release and recapture of *Cathartus quadricollis*

At the three sites where *C. quadricollis* adults were released, recovery was successful in collections of raisin coffee at 1, 2, and 7 weeks post-release (Fig. 3). The number of beetles recovered varied by site and time post-release, ranging from four to 162 adults per sampling date. The Kimura Lauhala site had relatively low recovery (≤10 individuals, ≤0.1% of total beetles released) of *C. quadricollis* at week 1

Fig. 2 Artificial bean experiment in the field (top) and close-ups of the artificial bean closed (bottom left) and open (bottom right)

post-release, and even fewer at week 2, and slightly higher at week 7 compared to week 2. Recovery at the Aikane Upper and Aikane Lower farms was >100 *C. quadricollis* (>1% of total beetles released) adults on all sampling dates post-release. The Aikane Upper site showed a constant recapture of approximately the same amount of *C. quadricollis* over time; whereas, the Aikane Lower site showed a slight downward trend over time in the number of *C. quadricollis* recaptured. Two teneral *C. quadricollis* adults were collected at the Aikane Upper farm indicating that reproduction occurred post-release. This reproduction likely occurred in the coffee raisin as no other suitable host plants were nearby. Additionally, the recovery of beetles after 7 weeks suggests that releasing *C. quadricollis* can result in establishment of populations in areas where they previously did not occur.

Molecular detection of scolytid prey

Fifty predator flat bark beetle adults were collected per landscape type (total $N = 200$) and analyzed for the presence or absence of scolytid prey DNA (Table 3). Both *H. hampei* and *X. compactus* DNA were detected in flat bark beetle individuals from the coffee-only landscape. Only *H. obscurus* was detected in predators from the macadamia nut-only landscape. *H. obscurus*, *H. hampei*, and *X. compactus* were detected in predators collected from coffee raisins in a mixed coffee/macadamia nut landscape. Only *H. obscurus* was detected in macadamia nut sticktights in the same mixed environment. *H. hampei* and *X. compactus* were detected in a predominantly *L. leucocephala* habitat.

Predation rates in sleeve cages and artificial berries

Sleeve cage study In general, augmentation of *C. quadricollis* in sleeve cages resulted in a decrease in *H. hampei* life stages, but results were variable and more or less pronounced depending on the farm (Fig. 4). Analysis of variance (ANOVA) showed that the effect of farm $F(2,27) = 3.209$, $p = 0.0562$ was nearly significant for *H. hampei* eggs, but not the effect of treatment $F(2,27) = 2.148$, $p = 0.1363$ nor treatment by farm interaction $F(4,27) = 1.048$, $p = 0.4012$. For larvae, the effects of treatment $F(2,27) = 6.699$, $p = 0.00434$, farm $F(2,27) = 20.374$, $p < 0.001$, and treatment by farm interaction $F(4,27) = 3.273$, $p = 0.026$ were all significant. For pupae, the effects of treatment $F(2,27) = 1.070$, $p = 0.3572$ and farm $F(2,27) = 0.8$, $p = 0.4599$ were not significant; the treatment by farm interaction was nearly significant $F(4,27) = 2.657$, $p = 0.0545$.

A post-hoc Tukey's HSD showed that the *H. hampei*-only treatment was significantly different from the 10 *C. quadricollis* and 40 *C. quadricollis* treatments for

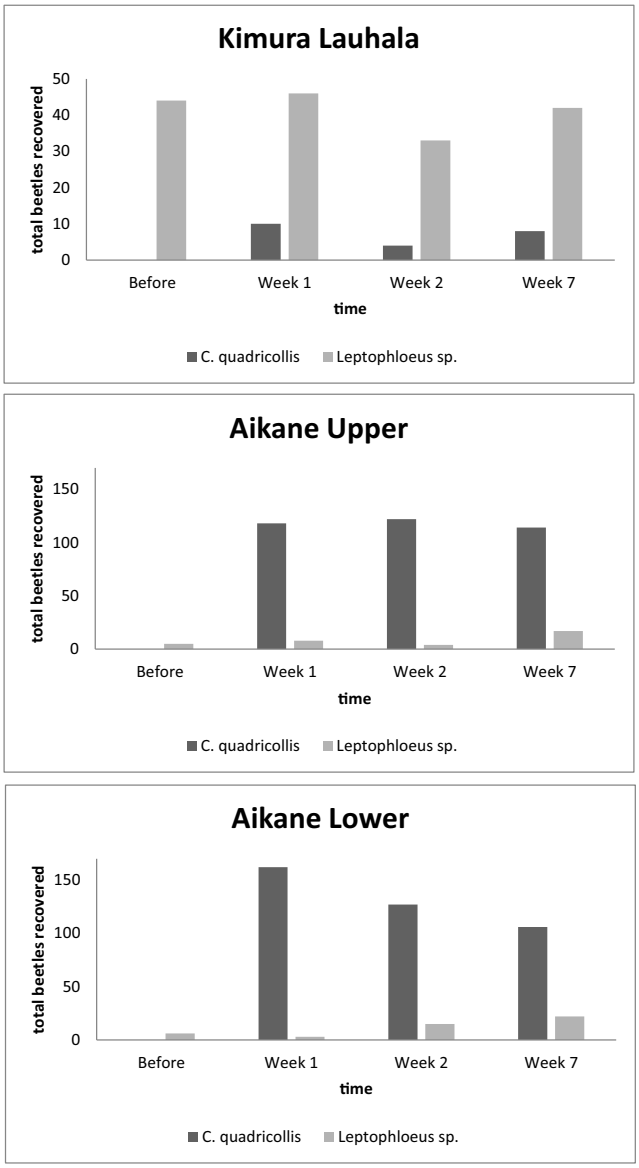

Fig. 3 Total number of flat bark beetles collected for each species at each collection date from each of the three mass release sites. Note: the vertical axis scale is different at Kimura Lauhala than Aikane Upper and Lower

H. hampei larvae. For *H. hampei* larvae, the Extension site was significantly different from the Old Ways and Mai Mahealani farms.

On a farm-by-farm analysis, there was no significance amongst treatments and life stages for the Extension nor Old Ways farms (Fig. 4). A numerical trend at the Extension farm was observed (i.e., a reduction in eggs and larvae in the 10 and 40 *C. quadricollis* treatments compared with the *H. hampei* only treatment). For the Mai Mahealani farm, treatments were significantly different for *H. hampei* larvae $F(2,9) = 7.161$, $p = 0.0138$ and pupae $F(2,9) = 6.949$, $p = 0.015$. Eggs had an observable numerical trend, but not a significant one. A post-hoc Tukey's HSD showed that for larvae, the *H. hampei*-only treatment was different from the 10 *C. quadricollis* and 40 *C. quadricollis* treatments. For pupae, the 40 *C. quadricollis* and 10 *C. quadricollis* treatments were significantly different from each other, and the *H. hampei*-only treatment was not significantly different from either (Fig. 4).

Artificial coffee berries In total, 156 artificial coffee berries containing 3120 eggs were placed on 13 different dates across six different coffee farms. Egg predation occurred in 147 of the 156 artificial berries placed. The grand mean (+SE) predation rate was 40.2 (±2.0) %, with rates ranging from 13.3–63.8% across the farm sites by date combinations (Table 4).

Discussion

Our studies showed that all *C. quadricollis* life stages and *Leptophloeus* sp. larvae and adults were found in coffee, macadamia nut and several other locally abundant plant hosts, indicating that these predators are reproducing in the same hosts where they are feeding. These host plants harbor a variety of insects, including other scolytine bark beetles. Follett et al. (2016) previously reported collecting few larvae and no pupae in coffee and speculated that coffee may not be a primary reproductive host for flat bark beetles. This points to the importance of collection techniques; dissection of coffee berries as in the present study, provides exact information on insects and life stages, whereas Berlese funnel sampling (Follett et al. 2016) biases sampling toward mobile stages, especially adults.

Molecular analysis of *C. quadricollis* and *Leptophloeus* sp. showed they are feeding on *H. hampei*, *H. obscurus* and

Table 3 Percentage of scolytid prey DNA detected in *C. quadricollis* and *Leptophloeus* sp. in various agricultural landscapes

Habitat	Sample Unit	N	% with *H. hampei* DNA	% with *H. obscurus* DNA	% with *X. compactus* DNA
Coffee	Raisins	50	62	0	6
Macadamia Nut	Sticktights	50	0	30	0
Mixed coffee / macadamia nut	Raisins	25	48	8	4
Mixed coffee / macadamia nut	Sticktights	25	0	24	0
L. leucocephala	Seed pods	50	14	0	24

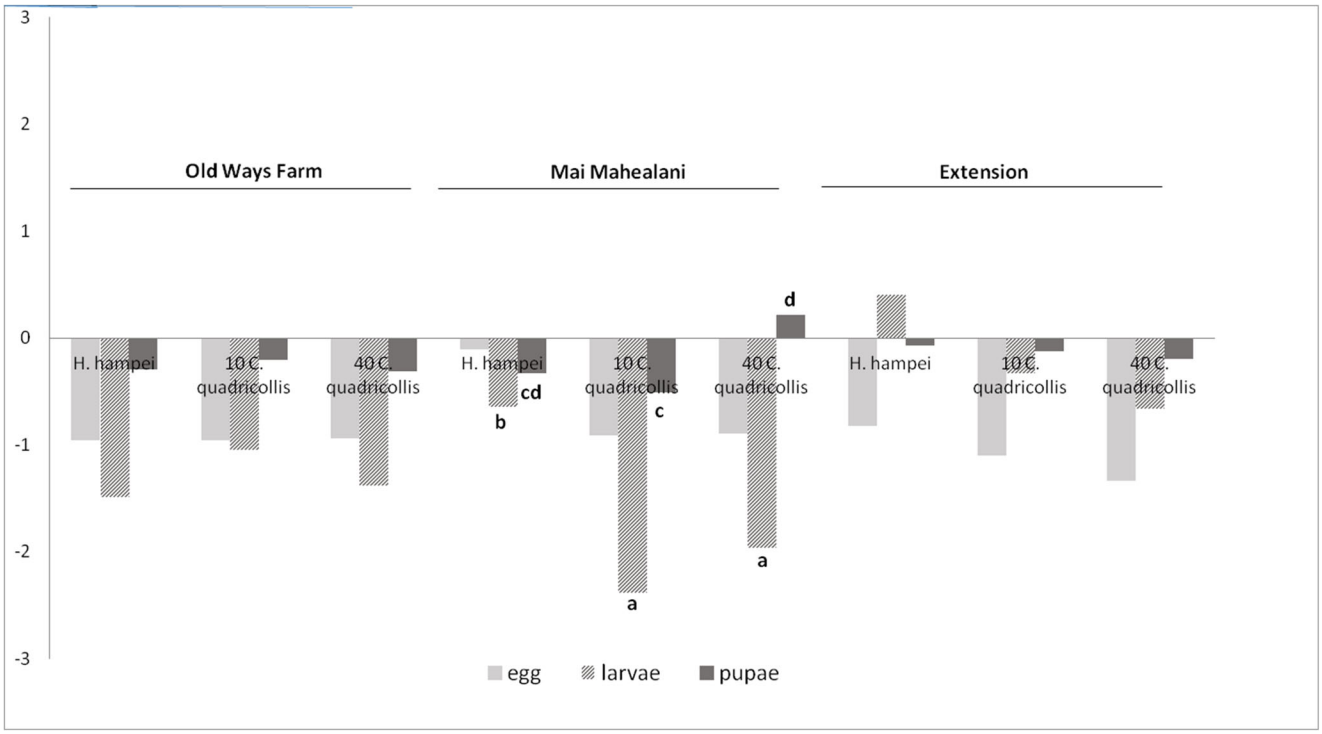

Fig. 4 Change in life stage values for each treatment per farm. Bars with different letters by field above them are significantly different from each other by a Tukey's HSD test ($p < 0.05$).

X. compactus in coffee, macadamia nut, and mixed coffee-macadamia nut farms. In coffee farms, predators appeared to only feed on the predominant prey, *H. hampei*, suggesting that movement among coffee and non-coffee habitats may be limited. Likewise, in macadamia nut farms, predators appeared to only feed on the predominant prey, *H. obscurus*. In mixed farms, all three prey items can be found in coffee berries (Greco and Wright 2012); whereas, only *H. obscurus* occurs in sticktight macadamia nuts. This was reflected in the molecular analysis of predator feeding (Table 3). *H. hampei* and *X. compactus* DNA was also found in predators collected from *L. leucocephala* pods. Since *X. compactus* feeds on many hosts in the landscape, but *H. hampei* is specific to coffee, this finding suggests that the flat bark beetle predators must have moved to *Leucaena* after feeding in coffee.

Table 4 Predation of *H. hampei* eggs in artificial coffee berries, 2017

Farm	Month	#Artificial Berries	# Artificial berries showing predation	# Eaten (mean + SE)[a]	% E(mean + SE) [a] aten
Lehuula	March	12	12	12.8 (0.9)	63.8 (4.5)
	April	12	12	2.7 (0.3)	13.3 (1.7)
	May	12	12	3.1 (0.8)	15.4 (4.1)
Middle Keei	March	12	12	9.9 (1.0)	49.6 (4.9)
	May	12	12	8.3 (0.7)	41.3 (3.6)
	July	12	8	5.6 (1.7)	27.9 (8.3)
Greenwell	May	12	12	12.2 (0.8)	60.8 (4.2)
	July	12	10	7.9 (1.6)	39.6 (8.2)
OK	June	12	12	7.1 (1.2)	35.4 (5.8)
	August	12	11	8.5 (1.3)	42.5 (6.6)
Rodeo	June	12	11	7.9 (1.3)	39.6 (6.7)
	August	12	12	12.1 (1.0)	60.4 (5.0)
Carnation Lei	July	12	11	6.6 (1.5)	33.0 (7.4)

[a] Calculated from number or percentage eaten out of 20 eggs placed in each artificial coffee berry

In previous laboratory studies, *C. quadricollis* and *Leptophloeus* sp. fed mainly on immature life stages (eggs, larvae, pupae) of *H. hampei* (Follett et al. 2016). However, predation rates in the sleeve cage experiments were highly variable and statistically significant only on the Mahealani Farm. These results may have been caused by variation in the number of *H. hampei* life stages in the infested coffee berries inside the sleeves at the start of the experiment. Ideally, predation rates could be estimated from naturally infested coffee berries as predators may be attracted to damaged berries. The sleeve cage study indicated this would be difficult statistically due to high variability in infestation rates. The artificial berry experiment using hollowed out wooden beads stocked with prey was an attempt to overcome the problem of unknown prey numbers. Thus far, only *H. hampei* eggs have been tested, but predation averaged 40.2% (1254 out of 3120 total eggs) across six farms on multiple dates. This suggests that flat bark beetle predators can have a significant impact on *H. hampei* numbers in raisin coffee.

Flat bark beetles have potential as augmentative biological control agents against *H. hampei* and *X. compactus* in coffee, and against *H. obscurus* in macadamia nut. In coffee, these predators are mainly attacking *H. hampei* in dried coffee left on the tree after harvest. It has been shown recently that raisins left on coffee trees have significantly higher CBB infestation than ground raisins, and ground raisins positively correlate with next season's CBB infestation (Johnson et al. 2019). In macadamia nut, these predators are mainly found in dried sticktight nuts in the tree. Therefore, the role of flat bark beetle predators in management of *H. hampei* and *H. obscurus* in coffee and macadamia nut, respectively, will be to suppress population growth in unharvested berries and nuts between seasons and in abandoned fields. *C. quadricollis* and *Leptophloeus* sp. can be raised inexpensively on a diet of cracked corn and cornmeal (Follett et al. 2016). Hawaii coffee growers were shown how to raise flat bark beetles on this diet and more than 250 'starter kits' with predators on food and rearing containers were given out during workshops and other outreach events (Follett et al. 2016). Many farmers are now periodically releasing home-grown predatory beetles on their farms to augment existing populations. Our finding that released *C. quadricollis* can be found in coffee fields for 7 weeks afterward indicates that augmentation will significantly increase numbers of predators in the field. To facilitate field augmentation, a commercial breeding station has been developed that uses a *C. quadricollis* aggregation pheromone (Pierce et al. 1988) to attract adults to food where they can multiply and disperse into the crop with minimal farmer input required (Follett, unpublished). For IPM in coffee, release of predators, or deploying breeding stations, should be timed to increase numbers during and after harvest when overripe and raisin coffee is most abundant. The actual impact that higher numbers of predators may have in coffee or macadamia nut farms is difficult to quantify due to the cryptic habits of their scolytine prey. Additionally, a multitude of environmental factors could affect predator success and establishment. The relatively high (40%) predation on *H. hampei* eggs in artificial berries placed in coffee fields suggests *C. quadricollis* and *Leptophloeus* sp. may be a substantial source of mortality. Future studies will examine predation on other *H. hampei* life stages using artificial berries. The exotic endoparasitoid *Phymasticus coffea* (Hymenoptera: Eulophidae) which has been released in Central and South American and other coffee production areas around the world (Aristizabal et al. 2004, 2016), is undergoing host range testing in quarantine in Hawaii for possible release. *P. coffea* attacks adult *H. hampei* as they bore into green berries, and thus, interference competition between flat bark beetles (which occur mostly in dried raisin coffee) and this parasitoid should not occur.

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