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Predation by flat bark beetles (Coleoptera: Silvanidae and Laemophloeidae) on coffee berry borer (Coleoptera: Curculionidae) in Hawaii coffee

Peter A. Follett [a,*], Andrea Kawabata [b], Robert Nelson [c], Glenn Asmus [a], Jen Burt [b], Kally Goschke [b], Curtis Ewing [d], Julie Gaertner [d], Eva Brill [a], Scott Geib [a]

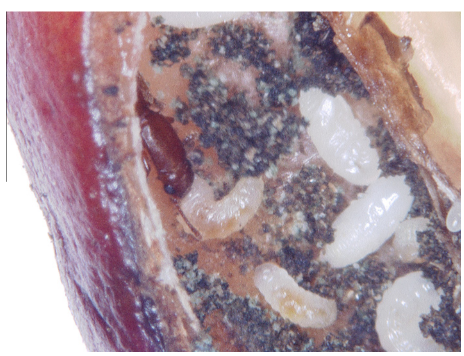
[a] U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS), Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, Hilo, HI 96720, United States
[b] University of Hawaii Cooperative Extension, College of Tropical Agriculture and Human Resources, Kealakekua, HI 96750, United States
[c] Lehu'ula Farms, Kealakekua, HI 96750, United States
[d] University of Hawaii at Hilo, Department of Biology, Hilo, HI 96720, United States

HIGHLIGHTS

- The flat bark beetles *Leptophloeus* sp. and *Cathartus quadricollis* feed on coffee berry borer (CBB) in Hawaii coffee.
- Adult and larval predators can feed on all CBB life stages.
- *C. quadricollis* was not susceptible to infection by the biopesticide *Beauveria bassiana* used for CBB control in coffee.
- These predators can be reared easily and inexpensively for augmentative releases.

GRAPHICAL ABSTRACT

Adult *Cathartus quadricollis* feeding on a coffee berry borer larva inside a coffee berry.



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ABSTRACT

Coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) is a serious pest of coffee worldwide and a new invasive pest in Hawaii. Adult flat bark beetles, mainly *Leptophloeus* sp. (75%) and *Cathartus quadricollis* (21%) (Coleoptera: Laemophloeidae and Silvanidae, respectively), were found inside CBB-infested coffee beans on the tree and molecular marker studies confirmed feeding on CBB. Research was conducted to better understand the ecology of these predators and explore ways to increase their role in suppressing CBB populations in coffee. Laboratory feeding assays demonstrated the capacity for adult and larval flat bark beetles to feed on all CBB life stages. The predators are widely distributed in the coffee growing areas on the island of Hawaii, but feed mainly in dried coffee on the tree rather than in ripening berries where initial crop damage occurs. Berlese funnel extraction of flat bark beetles from dried beans on the tree indicated that predator numbers can be relatively high (up to 23 adult predators per 150 bean sample). *C. quadricollis* was not susceptible to infection by the fungal biopesticide *Beauveria bassiana* which is used for field control of CBB in coffee. *Leptophloeus* sp. and *C. quadricollis* can be raised easily on a diet of cracked corn

* Corresponding author at: USDA-ARS-DKI USPBARC, 64 Nowelo St., Hilo, HI 96720, United States.
E-mail address: peter.follett@ars.usda.gov (P.A. Follett).

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and cornmeal. Augmentative releases of generalist flat bark beetle predators like *Leptophloeus* sp. and *C. quadricollis* may be a useful component of integrated pest management programs against coffee berry borer and other scolytine pests.

1. Introduction

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) is the most important pest of coffee worldwide, with damage exceeding US$500 million annually (Jaramillo et al., 2006). CBB was first discovered in Hawaii in 2010 on the Kona side of the island of Hawaii where there are about 800 small farms on 1170 ha, and it has since moved to the island of Oahu (Chapman et al., 2015). CBB is the greatest threat to Hawaii's coffee industry. Current levels of coffee infestation by CBB average 15–20%, which causes yield loss, reduced quality and price, and increased costs (Aristizabal et al., 2016). If left unchecked, CBB can infest >90% of coffee berries at harvest.

All CBB life stages develop inside the coffee berry (Aristizabal et al., 2016). Adult females bore into hardened developing green berries and excavate tunnels and galleries in which they lay their eggs. Females may lay >100 eggs in a single berry during a three week period. Inside the berry, CBB develops through six life stages: egg, larva (first and second instars), pre-pupa, pupa, and adult, during 1–2 months depending on temperature and hardness of the berry (Baker, 1999). The founding female remains with her progeny and does not leave the berry. Siblings can mate and CBB have a female-biased sex ratio of 10:1. Berry development requires 200–250 days from flowering to harvest, and therefore individual berries can support multiple generations of CBB (Baker et al., 1992). CBB will continue to breed in coffee berries as they ripen, senesce, and dry to the 'raisin' stage. Currently, management of CBB in Hawaii focuses on sampling and monitoring, monthly sprays of the biopesticide *Beauveria bassiana*, and sanitation (removal of all berries at the end of harvest) (Aristizabal et al., 2016). When harvest is completed, any remaining 'raisin' coffee not stripped from the trees may serve as a source of CBB for the subsequent crop (Vega et al., 2015).

Adult flat bark beetles, mainly the lined flat bark beetle *Leptophloeus* sp. (Coleoptera: Laemophloeidae) and the silvanid flat bark beetle *Cathartus quadricollis* (Guerin-Meneville) (Coleoptera: Silvanidae), were found inside CBB-infested coffee berries on trees in Hawaii. Molecular markers were used to verify predation by detecting the presence of CBB DNA in the gut of the flat bark beetles (Sim et al., 2016). In general, the ecology of most flat bark beetles (*sensu lato*) is poorly understood, but they are known to feed on small insects and mites under bark, and many groups feed on fungi (Thomas, 1993). Some species of flat bark beetles, including species of *Leptophloeus*, are found only in the galleries of scolytine bark beetles, and a few are stored grain pests, including *C. quadricollis* (the square-necked grain beetle) (Thomas, 1993). *C. quadricollis* has been collected in Colombia from infested coffee presumably feeding on CBB (Bustillo et al., 2002), *Leptophloeus* sp. near *punctatus* has been observed feeding on CBB larvae in Togo and Ivory Coast (Vega et al., 1999), and *Leptophloeus* sp. and *C. quadricollis* have been found on macadamia nut in Hawaii where they are likely feeding on another scolytine pest, *Hypothenemus obscurus* (F.), the tropical nut borer (Jones et al., 1998). Since the lifestyle of CBB is cryptic and its entire life cycle occurs inside the coffee berry, direct study of predators and predation by flat bark beetles is difficult.

Field studies were conducted to determine the geographic and within-plant distribution, abundance, and diversity of flat bark beetles in coffee. Laboratory studies were undertaken to assess flat bark beetle preference for feeding on various CBB life stages, and susceptibility of flat bark beetles to infection by the biopesticide *Beauveria bassiana* used for CBB control.

2. Materials and methods

2.1. Distribution and abundance

Five farms were selected along an elevation gradient (from 100 to 800 m) and infested harvest-ripe coffee berries were sampled weekly to determine if predator numbers increased in response to increasing coffee berry borer infestations as the season progressed. After sampling for seven months and dissection of 2800 CBB-infested berries, practically no flat bark beetles were collected and it was determined that flat bark beetles do not prefer this crop maturity stage. All studies from that point on focused on raisins (dried berries on the tree), where flat bark beetle predators were more commonly found.

A survey of coffee farms on the island of Hawaii was conducted to determine the distribution of flat bark beetles. CBB-infested coffee raisins were sampled during the coffee harvest period of September to December 2014 in an attempt to determine presence or absence of flat bark beetles. A total of 160 collections were made from 80 coffee farms. Raisins were sampled from coffee trees by walking along rows and systematically sampling raisins from branches with raisins or, when rows were not present by sampling during a zigzag walk through the coffee trees, until a Whirl Pak bag (118 ml, 7.6 × 18.4 cm, Zefon International, Ocala, FL) was filled. A Whirl Pak bag was filled with an average of 150 raisins for every 1 ha of coffee. Each sample of raisins was placed in a Berlese funnel for 24 h and flat bark beetles emerging from the funnel were counted and identified.

2.2. Insect rearing

The two main species of predator beetles collected from field sampling were an undescribed species of lined flat bark beetle *Leptophloeus* sp. and the silvanid flat bark beetle *Cathartus quadricollis*. Laboratory and field studies thus focused on these two species. Adult *Leptophloeus* sp. and *C. quadricollis* were collected from multiple coffee farms in Captain Cook, Hawaii and reared in the laboratory on a diet of cracked corn and cornmeal (4:1) in the dark at 24 °C (±2 °C). Additional beetles were regularly collected from the field and added to the colonies to ensure availability of adults and larvae for laboratory experiments. Predator colonies had overlapping generations, and therefore adult and larval predators used in experiments were of indeterminate age. Coffee berry borer life stages used in experiments were mainly obtained by dissecting infested coffee raisins from the field. Coffee berry borer was also reared in the laboratory using an artificial diet modified from Villacorta and Barrera (1993) and Vega et al. (2011) to supplement field-collected beetles as needed.

2.3. Feeding preference bioassays

Laboratory studies were conducted to examine feeding preferences of flat bark beetle adults and larvae for various CBB life stages. In no choice experiments, three flat bark beetles were transferred to a Petri dish (100 × 15 mm) that contained 20 prey items on filter paper (90 mm diam. white) moistened with 1 ml distilled water and sealed with parafilm. Predators were the adult or late larval stage of *Leptophloeus* sp. or *C. quadricollis* and prey were CBB eggs, second instar larvae, pupae or adults. After 24 h in the dark at 24 °C (±2 °C), predators were removed and any remaining CBB prey items were assessed and counted. Eggs and larvae were typically entirely eaten by the predators, whereas pupae were only partially consumed, and adults were maimed (e.g. missing legs). If the prey item was missing (egg, larva) or dead with noticeable wounds (pupa, adult) it was recorded as 'consumed'. In choice experiments, experimental conditions were identical except three predators were placed with 40 prey items, ten each of each CBB life stage (eggs, larvae, pupae and adults). Data on numbers consumed were subjected to analysis of variance and means separations were performed using a post-hoc Tukey's test (SAS, 2014).

2.4. Coffee berry maturity effect

A study was conducted to quantify the effect of coffee berry maturity stage on infestation level by flat bark beetles. Coffee trees have indeterminate flowering in Hawaii and therefore all maturity stages of berries can occur on the same branch. One hundred CBB-infested ripe, overripe and raisin stage coffee berries were collected from each of three trees on five farms. Each maturity stage by tree by farm sample was placed in a separate a Berlese funnel and all flat bark beetles emerging from the funnels at 24 h were counted and identified. Data for numbers of flat bark beetles were log transformed and subjected to analysis of variance and means separations were performed using a Tukey's test.

2.5. Tree versus ground berries

Flat bark beetles are difficult to find in CBB-infested coffee berries on the ground. A study was conducted to quantify the effect of coffee berry location (tree versus ground) on flat bark beetle occurrence. Seventeen samples were collected from nine farms by collecting CBB-infested raisins both in the tree and recently fallen on the ground during a random walk. Multiple samples were taken from several large farms by sampling different areas of the farm. Sample sizes ranged from 50 to 200 raisins per sample with a mean (±SE) sample size of 123 ± 7.4. Each sample was placed in a separate Berlese funnel and all flat bark beetles emerging from the funnels at 24 h were counted and identified. Data for numbers of flat bark beetles were log transformed and subjected to analysis of variance.

2.6. Beauveria bassiana dip bioassay

A laboratory study was conducted to determine relative levels of susceptibility between CBB and *Cathartus quadricollis* to infection by *Beauveria bassiana* formulated in BotaniGard ES (11.3% AI, Laverlam International Corp., Butte, MT), which is used for field control of CBB in Hawaii. *C. quadricollis* was used as a proxy for flat bark beetles in general. Diet-reared *C. quadricollis* and CBB were used to minimize previous exposure to *B. bassiana*. A dip bioassay was performed using a dilution series of the commercial product. Treatments were a 0.5×, 1.0×, 2.0× of the field rate of BotaniGard (field rate 0.25 L/100 L) plus a surfactant (Widespread Max, field rate 0.04 L/100 L). Specifically, the 2.0× field rate solution contained 2.5 ml BotaniGard plus 0.39 ml Widespread Max in a 1 L final mixture. For each treatment, 40 adult CBB or *C. quadricollis* were transferred to a 29.6 ml plastic cup (Dart conex complements portion container, Mason, MI), a 10 ml BotaniGard suspension was added to the cup, and then the cup was capped and inverted repeatedly for three seconds. After dipping, the contents of the cup were poured onto sterile paper towels and treated beetles were transferred with a paint brush to Petri dishes (100 × 15 mm) containing filter paper (90 mm diam. white) moistened with 1 ml distilled water and a small amount of food (20 grains of cracked corn for *C. quadricollis*; 5 ml artificial diet for CBB) and sealed with parafilm. Individual Petri dishes with treated beetles were placed inside plastic Ziploc bags to prevent escapes. Control insects for both species were dipped in sterile distilled water only and processed and held similarly. The dip test was replicated four times at each dose for each species. At 10 days, all beetles were scored as alive, dead or moribund by prodding with a fine tipped paint brush, and dead or moribund beetles were inspected for *B. bassiana* colony growth under a stereomicroscope. Data for percentage mortality and *B. bassiana* infection were subjected to analysis of variance and means separations were performed using a Tukey's test.

3. Results

3.1. Distribution and abundance

Leptophloeus sp. and *Cathartus quadricollis* were the most common predators found feeding inside CBB-infested coffee berries collected from coffee trees in our survey, accounting for 75% and 21% of captures respectively (Table 1). Adult predators, but not larvae, were collected in the survey. Several other species of potential predators were also encountered albeit rarely (Table 1). Since the only prey item in coffee berries in Hawaii is CBB, it is assumed that these other beetles may have been predating on CBB, although this was not determined by DNA analysis of gut contents. *Corticeus pratermissus* is listed as a bark beetle predator in Wegensteiner et al. (2015). *Cryptamorpha desjardinsii* is reportedly predaceous in the larval stage (Thomas, 1993). *Silvanus bidentatus* is reported as a predator of *Tomicus* bark beetles (Lieutier et al., 2015). *Aneorps* sp. is a monotomid and many species within this family are found in the galleries of scolytine bark beetles and are thought to be predaceous (Sen Gupta, 1988; Wegensteiner et al., 2015). *Xylolestes laevior* is a laemophloeid and a number of species in this family occur in the galleries of scolytine bark beetles as well (Thomas, 1993).

Leptophloeus sp. and *C. quadricollis* were found at all elevations (100–800 m) and in all the coffee farming areas sampled (Fig. 1). Repeated sampling resulted in finding one or the other or both of these predators on all coffee farms sampled. The numbers of adult flat bark beetles collected from a sample of 150 dried berries ranged from 0 to a high of 23 (Fig. 2).

Table 1
Predatory beetles collected during three months using Berlese funnels from samples of CBB-infested dried coffee beans ('raisins') picked from the coffee tree.

Species	Family	No. captured	Percentage captures[a]	Percentage samples[b]
Leptophloeus sp.	Laemophloeidae	435	76.6	59.0
Cathartus quadricollis	Silvanidae	118	20.8	24.6
Corticeus praetermissus	Tenebrionidae	1	0.2	0.7
Cryptamorpha desjardinsii	Silvanidae	7	1.2	3.7
Aneurops sp.	Monotomidae	5	0.9	3.7
Xylolestes laevior	Laemophloeidae	1	0.2	0.7
Silvanus bidentatus	Silvanidae	1	0.2	0.7

[a] Percentage of overall beetle captures (No. captured/total No. captured).
[b] Percentage of samples containing each species; 20% of samples contained both *Leptophloeus* sp. and *C. quadricollis*.

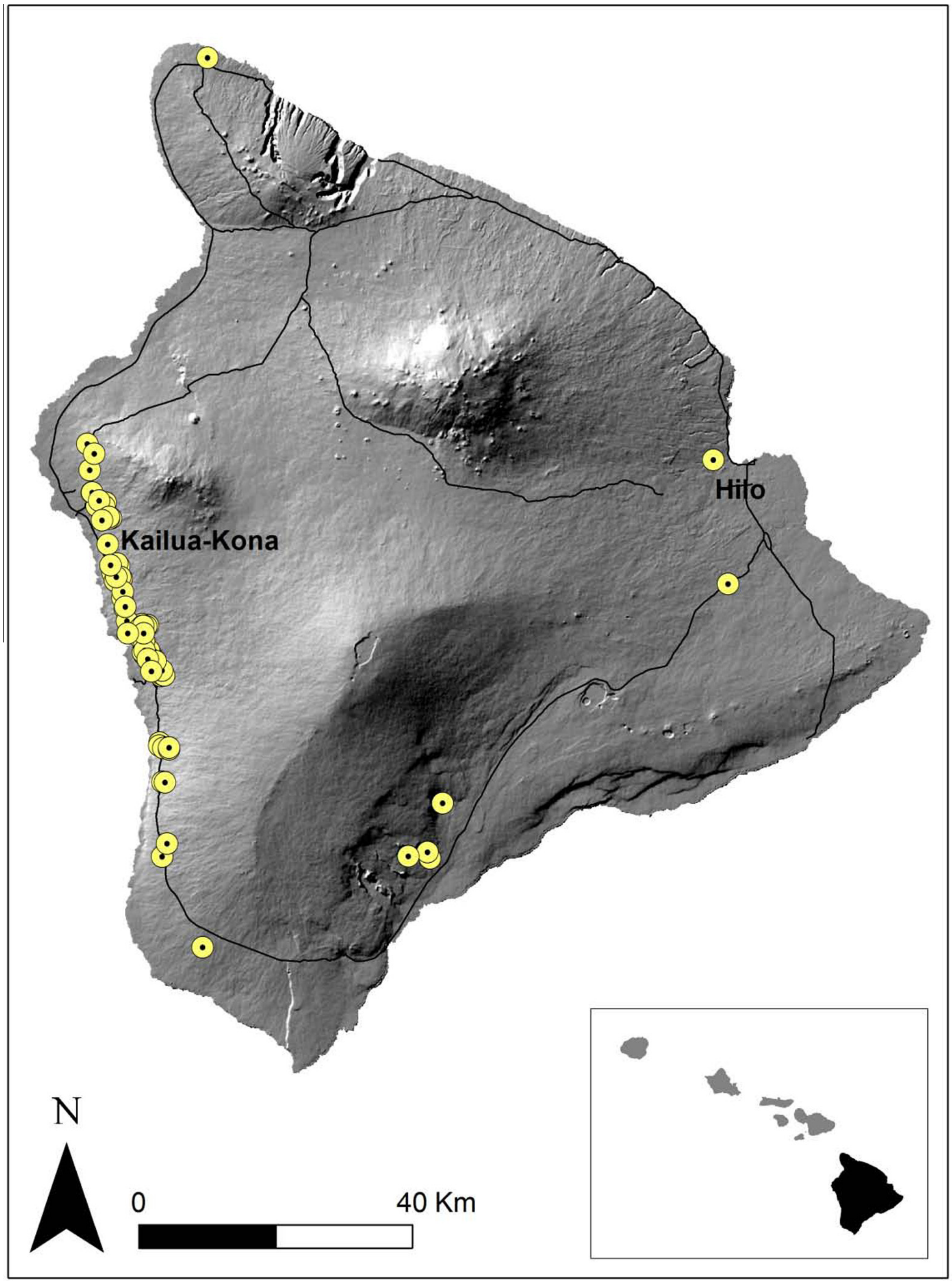

Fig. 1. Coffee farms on the island of Hawaii surveyed for flat bark beetle predators.

3.2. Feeding preference bioassays

In no choice laboratory feeding studies, where predators were presented with one CBB life stage at a time (20 prey items per 3 predators), percent prey consumption was significant for the effect of predator species ($F_{1,1} = 19.4$, $P < 0.0001$), the predator species by predator life stage interaction ($F_{1,1} = 17.5$, $P < 0.0001$), CBB prey stage ($F_{13,3} = 36.6$, $P < 0.0001$), and the predator stage by CBB prey life stage interaction ($F_{3,3} = 3.4$, $P = 0.02$). Overall, *C. quadricollis* ate significantly more CBB than *Leptophloeus* sp. (Figs. 3 and 4). *C. quadricollis* adults ate significantly more CBB than *C. quadricollis* larvae ($F_{1,1} = 13.0$, $P < 0.001$). Adult *C. quadricollis* ate significantly more CBB eggs (77.5%), larvae (55.0%) and pupae (53.0%) than adults (15.5%) ($P < 0.05$); and larval *C. quadricollis* ate more CBB eggs (63.0%) than larvae (31.5%), pupae (19.0%) and adults (6.0%) ($P < 0.05$). Although predator feeding on CBB adults in no choice tests generally involved eating the legs, in one instance a *C. quadricollis* larvae had eaten a significant portion of the body of a teneral CBB adult. Unlike *C. quadricollis*, *Leptophloeus* sp. larvae ate more CBB than *Leptophloeus* sp. adults ($F_{1,1} = 5.4$, $P < 0.001$) (Fig. 4). Larval *Leptophloeus* sp. ate significantly more CBB eggs (74.0%) than larvae (22.5%), pupae (19.0%) and adults (0.9%) ($P > 0.05$); and adult *Leptophloeus* sp. ate significantly more CBB eggs (32.5%) and larvae (29.0%) than pupae (8.5%) or adults (0%) ($P < 0.05$).

In the choice tests, where predators were presented all CBB life stages simultaneously, percent prey consumption was significant for the effect of predator species ($F_{1,1} = 15.6$, $P = 0.0002$), the predator species by predator life stage interaction ($F_{1,1} = 4.3$, $P = 0.04$),

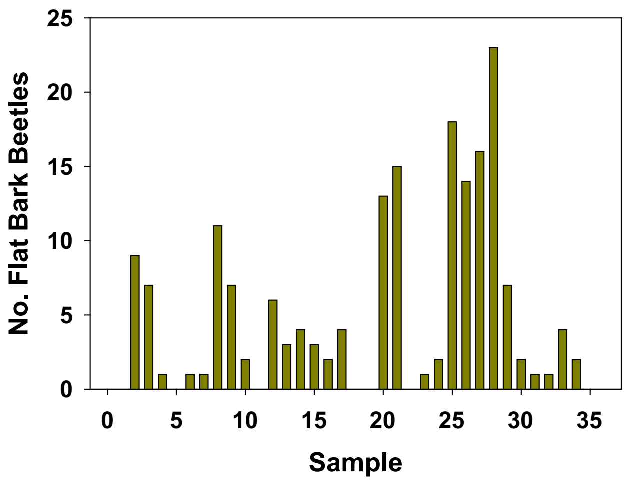

Fig. 2. Total number of flat bark beetles collected per 150 raisin sample from each of 35 coffee farms.

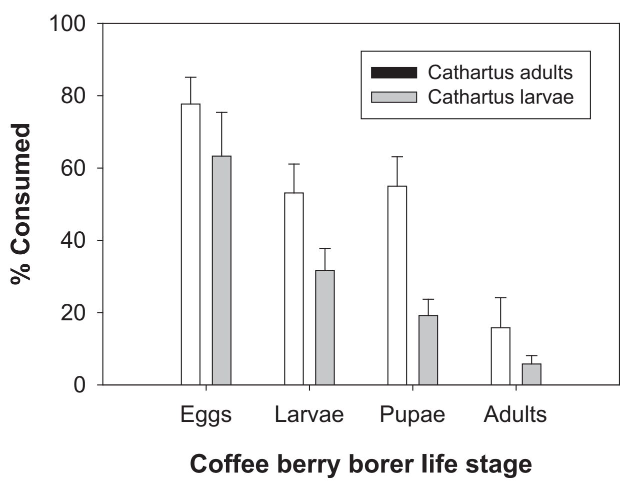

Fig. 3. No choice bioassay: average consumption (mean% ± SE) by *Cathartus quadricollis* adults and larvae of coffee berry borer life stages when presented independently.

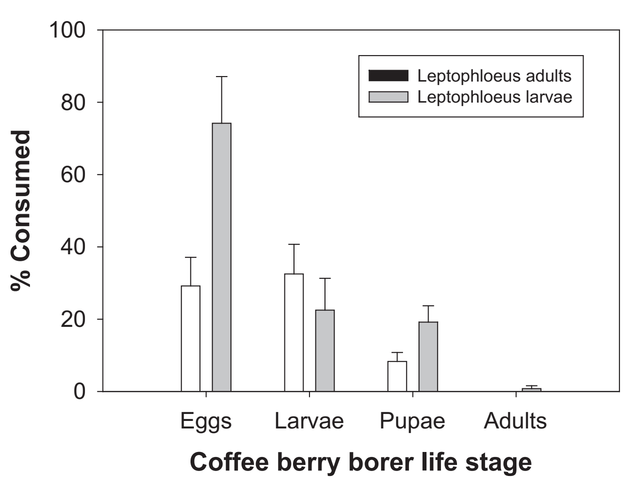

Fig. 4. No choice bioassay: average consumption (mean% ± SE) by *Leptophloeus* sp. adults and larvae of coffee berry borer life stages when presented independently.

CBB prey stage ($F_{3,3}$ = 35.6, P < 0.0001), and the predator species by CBB prey life stage interaction ($F_{13,3}$ = 5.5, P = 0.002). Overall, *C. quadricollis* ate significantly more CBB than *Leptophloeus* sp. (36.3% versus 18.8% of prey items) (Figs. 5 and 6). *C. quadricollis* adults and larvae ate equal numbers of CBB life stages ($F_{1,1}$ = 3.4, P = 0.07). Adult *C. quadricollis* ate significantly more CBB eggs (78.3%) than larvae (38.3%), and significantly more larvae than pupae (6.7%) and adults (0.0%) (P < 0.05). Larval *C. quadricollis* ate significantly more CBB eggs (75.0%) and larvae (71.7%), than pupae (20.0%) and adults (0%) (P < 0.05). *Leptophloeus* sp. adults and larvae ate equal numbers of CBB ($F_{1,1}$ = 1.4, P = 0.24). Adult *Leptophloeus* sp. ate significantly more CBB eggs (48.3%) than larvae (16.7%) and adults (0%) (P < 0.05), and an intermediate number of pupae (25.0%). Larval *Leptophloeus* sp. ate significantly greater numbers of CBB eggs (36.7%) than adults (0%) (P < 0.05), and an intermediate number of larvae (20%) and pupae (8.3%).

The often higher consumption rates of eggs by predators may be due to the smaller size of eggs compared to the other life stages. Coffee berry borer eggs weighed on average 0.047 mg, whereas the larvae, pupae and adults used in the tests weighed on average 0.46, 0.49 and 0.39 mg, respectively, or approximately 10 times more. Thus, handling time for eggs may have been less than the other larger life stages. CBB adults suffered the lowest mortality probably due to their heavier sclerotization and higher mobility than the other stages. *C. quadricollis* generally ate more CBB than *Leptophloeus* sp., probably due to its larger size. Adult *C. quadricollis*

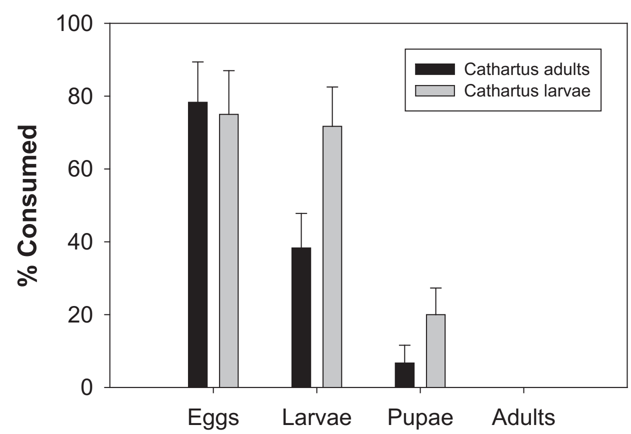

Fig. 5. Choice bioassay: average consumption (mean% ± SE) by *Cathartus quadricollis* adults and larvae of coffee berry borer life stages when presented simultaneously.

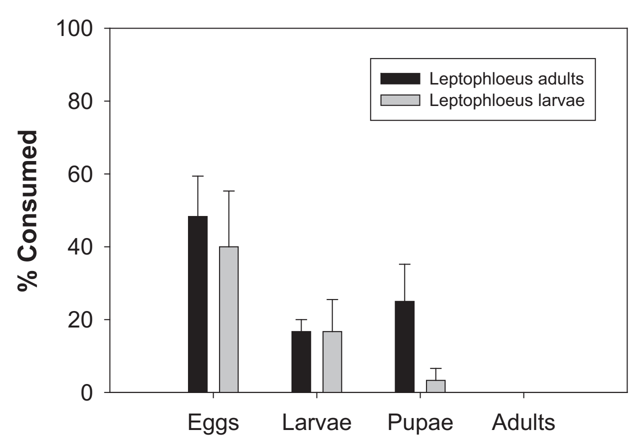

Fig. 6. Choice bioassay: average consumption (mean% ± SE) by *Leptophloeus* sp. adults and larvae of coffee berry borer life stages when presented simultaneously.

weighed on average 0.70 mg, more than twice the average weight of *Leptophloeus* sp. adults which was 0.33 mg. In choice tests, *C. quadricollis* adults ate 78.3% of CBB eggs and 38.3% of larvae compared with 48.3% and 16.7% respectively for *Leptophloeus* sp.

3.3. Maturity stage and location

The effect of coffee berry maturity stage on numbers of predators in CBB-infested coffee berries was significant ($F_{2,41} = 7.4$, $P < 0.002$). Coffee raisins were the most preferred habitat for *Leptophloeus* sp. and *C. quadricollis* adults, followed by overripe berries; predators were rare in ripe berries (Fig. 7). The location of coffee raisins was also important; *Leptophloeus* sp. and *C. quadricollis* adults were more commonly found in raisins on the tree than in newly dropped raisins on the ground ($t_{1,33} = 4.2$, $P < 0.05$) (Fig. 8).

3.4. Beauveria bassiana dip bioassay

In the *Beauveria* dip bioassay with CBB and *C. quadricollis*, percentage mortality was not significant for the effect of species ($F_{1,3} = 0.001$, $P < 0.001$), but was significant for the effect of concentration ($F_{3,31} = 3.4$, $P < 0.04$) and the species by concentration interaction ($F_{6,31} = 3.1$, $P < 0.05$). Percentage infection by *B. bassiana* was significant for the effect of species ($F_{1,3} = 22.9$, $P < 0.001$), and marginally not significant for the effect of concentration ($F_{3,31} = 2.7$, $P < 0.06$) and the species by concentration interaction ($F_{6,31} = 2.7$, $P < 0.06$) (Table 2). Percentage mortality in CBB increased as the BotaniGard treatment concentration increased from 0.5× to 2.0× the field rate, and *B. bassiana* infection was visible in 55–75% of dead beetles in the BotaniGard treatments. Percentage mortality was variable for *C. quadricollis*, but no *B. bassiana* infection was observed in dead beetles in the BotaniGard treatments (Table 2).

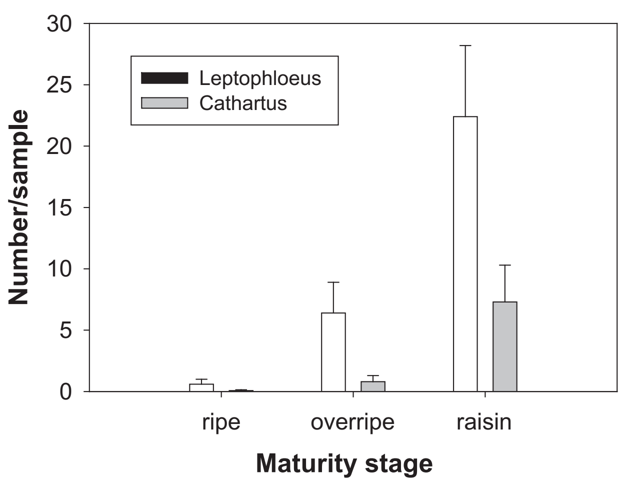

Fig. 7. Mean number (±SE) of flat bark beetles from samples of berries of different maturity stage (100 berries/sample).

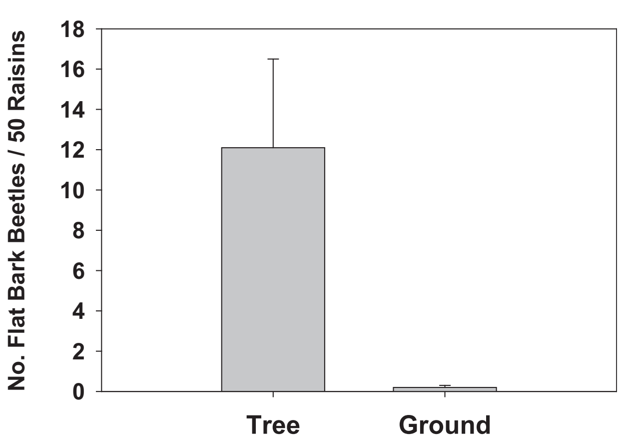

Fig. 8. Mean (±SE) number of flat bark beetle predators (*Leptophloeus* sp. and *C. quadricollis*) per 50 coffee berries sampled from the ground or from the tree (No. farms = 15).

Table 2
Susceptibility of coffee berry borer and *Cathartus quadricollis* to formulated *Beauveria bassiana* (BotaniGard ES) using a dip bioassay (mean + SE).

Field rate	No. tested	Coffee berry borer		No. tested	*Cathartus quadricollis*	
		Mortality (%)[a]	*B. bassiana* infection (%)		Mortality (%)	*B. bassiana* infection (%)
Control	154	5.8 (2.2)a	5.8 (2.2)a	149	5.3 (2.6)a	0
0.5	151	28.5 (6.1)ab	15.7 (3.1)a	158	32.5 (15.3)a	0
1.0	157	32.0 (22.9)ab	23.8 (16.5)a	156	10.9 (8.4)a	0
2.0	154	68.0 (9.5)b	41.5 (6.6)a	151	11.2 (6.0)a	0

[a] Means within a column followed by the same letter are not significantly different by a Tukey's test ($P > 0.05$).

4. Discussion

Our study showed that *Leptophloeus* sp. and *Cathartus quadricollis* are the most common predators inside CBB-infested coffee berries in coffee trees. Laboratory feeding assays demonstrated the capacity for adults and larvae of both species to feed on all life stages of CBB. Molecular marker studies confirmed that *C. quadricollis* and *Leptophloeus* sp. adults collected from dried berries on trees in the field are feeding on CBB (Sim et al., 2016). These predators are widely distributed in the coffee growing areas on the island of Hawaii, but feed mainly in dried coffee on the tree rather than in ripening berries where crop damage occurs. Predator numbers can be quite high, with up to 23 adult predators collected from a sample of 150 dried berries. The predators do not appear to be susceptible to the fungal biopesticide *B. bassiana* which is widely used for field control of CBB in coffee. Unlike other coffee growing areas around the world, flat bark beetles are the most common natural enemies of CBB in Hawaii.

Primarily adult flat bark beetles have been collected in coffee raisins, with relatively few collections of larvae and no collections of pupae. This suggests that flat bark beetle reproduction in CBB-infested coffee berries may be limited. The search for immature life stages of flat bark beetles in the field is ongoing. Recent studies have shown that all life stages of *C. quadricollis* and *Leptophloeus* sp. can be found in macadamia nut (*Macadamia integrifolia*), and in the seed pods of *Leucaena leucocephala* and several other leguminous trees, which are all common plants in the coffee landscape in Hawaii (Brill and Follett, unpublished data). These host plants harbor a variety of insects, including other scolytine pests, such as tropical nut borer and black twig borer, *Xylosandrus compactus* (Eichoff). Additional molecular markers are being developed to study patterns of flat bark beetle predation on other scolytine bark beetles in the coffee landscape. The life history of *C. quadricollis* and *Leptophloeus* sp. in Hawaii may be linked to these alternate hosts where development and reproduction occurs. The absence or shortage of reproductive host plants for these predators may explain why they are not more common in other coffee growing regions. Movement patterns from these alternate hosts into coffee should be studied to determine if there are ways to enhance immigration.

Natural enemies of CBB found in other coffee growing regions do not occur or are not common in Hawaii. Parasitoids used in augmentative release programs in other countries such as *Phymasticus* *coffea* and *Cephalonomia stephanoderis* are not present in Hawaii, and the predatory thrips *Karnyothrips flavipes* is present but is rarely seen in coffee. Classical biological control using parasitoids should be explored but may be hampered in practicality by the large number of native *Xyleborus* bark beetles in Hawaii that must be considered for non-target effects during host specificity testing (Aristizabal et al., 2016).

The results of our studies with flat bark beetle predators suggest their possible use in augmentative biological control. These predators are mainly attacking CBB in dried coffee left on the tree after harvest. Therefore, their role in CBB management will be to suppress population growth in unharvested coffee between seasons and in abandoned coffee. *Cathartus quadricollis* and *Leptophloeus* sp. can be raised inexpensively on a diet of cracked corn and cornmeal. Hawaii coffee growers were shown how to raise flat bark beetles on this diet and many farmers are now periodically releasing home-grown predatory beetles on their farms to augment existing populations. An aggregation pheromone has been identified for *C. quadricollis* (Pierce et al., 1988) and we are exploring ways to use pheromone and kairomone lures to draw greater numbers of the predators into coffee fields. A pheromone for the more common predator *Leptophloeus* sp. has not been identified yet. Augmentative release of predatory flat bark beetles that are easily reared in large numbers like *Leptophloeus* and *C. quadricollis* could be a useful component of an integrated pest management program for other scolytine pests as well.

Video

A video of a *C. quadricollis* adult chewing on a CBB larva in a CBB-infested coffee bean collected from the field can be viewed here: https://dx.doi.org/10.6084/m9.figshare.3486674.v1.

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References

Aristizabal, L.T., Bustillo, A.E., Arthurs, S.P., 2016. Integrated pest management of coffee berry borer: strategies from Latin America that could be useful for coffee farmers in Hawaii. Insects 7, 24. http://dx.doi.org/10.3390/insects7010006, 6.

Baker, P., 1999. The Coffee Berry Borer in Colombia. DFID-Cenicafe CABI Bioscience IPM for Coffee Project (CNTR 93/1536A). Cenicafe, Chinchina, Colombia, pp. 154.

Baker, P., Barrera, J.F., Rivas, A., 1992. Life-history studies of the coffee berry borer (*Hypothenemus hampei*, Scolytidae) on coffee trees in southern Mexico. J. Appl. Entomol. 29, 656–662.

Bustillo, A.E., Cardenas, R., Posada, F.J., 2002. Natural enemies and competitors of *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) in Colombia. Neotrop. Entomol. 31 (4), 635–639.

Chapman, E.G., Messing, R.M., Harwood, J.D., 2015. Determining the origin of the coffee berry borer invasion of Hawaii. Ann. Entomol. Soc. Am. 108 (4), 585–592.

Jaramillo, J., Borgemeister, C., Baker, P., 2006. Coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): searching for sustainable control strategies. Bull. Entomol. Res. 96, 223–233.

Jones, V.P., Finson, N.N., Richardson, M.S., 1998. Tropical nut borer natural enemies and koa seedworm management update. In: Proc. 38th Annual Conference of the Hawaiian Macadamia Nut Assoc., pp. 5–9.

Lieutier, F., Langstrom, B., Faccoli, M., 2015. The genus *Tomicus*. In: Vega, F.E., Hofstetter, R.W. (Eds.), Bark Beetles: Biology and Ecology of Native and Invasive Species. Academic Press, New York, pp. 371–426.

Pierce, H.D., Pierce, A.M., Johnson, B.D., Oelschlager, A.C., Borden, J.H., 1988. Aggregation pheromone of square-necked grain beetle, *Cathartus quadricollis* (Guer.). J. Chem. Ecol. 14, 2169–2184.

SAS Institute, 2014. JMP User's Guide. SAS Institute, Cary, North Carolina, USA.

Sen Gupta, T., 1988. Review of the genera of the Rhizophagidae (Clavicornia: Coleoptera) of the world. Memoirs of the Zoological Survey of India, vol. 17, pp. 58.

Sim, S., Yoneishi, N.M., Brill, E., Geib, S.M., Follett, P.A., 2016. Molecular markers detect cryptic predation on coffee berry borer (Coleoptera: Curculionidae) by silvanid and laemophloeid flat bark beetles (Coleoptera: Silvanidae, Laemophloeidae) in coffee beans. J. Econ. Entomol. 109, 100–105.

Thomas, M., 1993. The flat bark beetles of Florida (Coleoptera: Silvanidae, Passandridae, Laemophloeidae). In: Arthropods of Florida and Neighboring Land Areas, Florida Dept. Agric. Consum. Serv. Contribution No. 789, vol. 15, p. 101.

Vega, F.E., Mercadier, G., Damon, A., Kirk, A., 1999. Natural enemies of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) in Togo and Ivory Coast, and additional entomofauna associated with coffee beans. Afr. Entomol. 7, 243–248.

Vega, F.E., Kramer, M., Jaramillo, J., 2011. Increasing coffee berry borer (*Hypothenemus hampei*; Coleoptera: Curculionidae: Scolytinae) female density in artificial diet increases fecundity. J. Econ. Entomol. 104, 87–93.

Vega, F.E., Infante, F., Johnson, A.J., 2015. The genus *Hypothenemus*, with emphasis on *H. hampei*, the coffee berry borer. In: Vega, F.E., Hofstetter, R.W. (Eds.), Bark Beetles: Biology and Ecology of Native and Invasive Species. Academic Press, New York, pp. 427–494.

Villacorta, A., Barrera, J.F., 1993. Nova dieta meridica para criacao de *Hypothenemus hampei* (Ferrari, 1867). An. Soc. Entomol. Bras. 22, 405–409.

Wegensteiner, R., Werelinger, B., Herrman, M., 2015. Natural enemies of bark beetles: predators, parasitoids, pathogens, and nematodes. In: Vega, F.E., Hofstetter, R.W. (Eds.), Bark Beetles: Biology and Ecology of Native and Invasive Species. Academic Press, New York, pp. 247–304.