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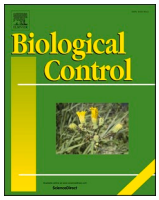

A predator breeding station for augmentative biological control of scolytine crop pests

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HIGHLIGHTS

- The square-necked grain beetle, *Cathartus quadricollis* is a predator of coffee berry borer and tropical nut borer in Hawaii.
- A predator breeding station was developed using a screen enclosure, lure, and food (corn) for augmentation biological control.
- Adult predators are attracted to breeding stations where they reproduce and multiply on the provided food and disperse back into the crop.
- Stocking with 100 *C. quadricollis* resulted in production and dispersal of about 10,000 adults per station over a four-month period.

GRAPHICAL ABSTRACT

Adult *Cathartus quadricollis* feeding on cracked corn in a breeding station.

ARTICLE INFO

Keywords:
Cathartus quadricollis
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ABSTRACT

The square-necked grain beetle, *Cathartus quadricollis* (Coleoptera: Silvanidae), is a predator of two significant crop pests in Hawaii: the coffee berry borer, *Hypothenemus hampei*, in coffee and the tropical nut borer, *H. obscurus* (Coleoptera: Curculionidae: Scolytinae) in macadamia nut. *C. quadricollis* is also a stored grain pest and is known to respond to its aggregation pheromone (i.e., quadrilure). Field tests were conducted in a macadamia nut orchard using sticky traps to identify the best lure dispenser and trap color based on captures of adult *C. quadricollis*. When quadrilure was released in different dispenser types, in combination with a fungal volatile blend released from a pouch, we found that a membrane-type quadrilure release dispenser (wafer) was superior to a red septa, gray septa, or open vial dispenser in attracting *C. quadricollis*. In subsequent tests, the combination of a quadrilure lure and a lure releasing a blend of fungal volatiles caught more *C. quadricollis* than the quadrilure lure alone. Also, we found that black sticky traps caught more *C. quadricollis* than did blue, green, red, yellow, white, or clear sticky traps. A predator breeding station consisting of a screened and sheltered enclosure containing food (250 g of cracked corn: cornmeal [4:1, w:w] mix) and the membrane-type quadrilure lure was developed to augment predator numbers in coffee and macadamia fields. In the laboratory, stocking a breeding station with 100 *C. quadricollis* resulted in production and dispersal of about 10,000 adults per station over a four-month period at 25 °C. In field tests, use of the quadrilure lure plus a fungal volatiles lure or the quadrilure lure alone were placed in breeding stations in a coffee field to attract naturally occurring wild adults for a five-

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week period, then returned to the laboratory where they both produced roughly 3,000 adults per station over a five-month period. The predator breeding station was an efficient way to multiply *C. quadricollis* numbers in the field and could be used to augment biological control of the coffee berry borer in coffee and tropical nut borer in macadamia nut.

1. Introduction

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), is the most serious insect pest of coffee worldwide, with crop loss 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 coffee farms on 1170 ha, but it has since spread to the other islands (Chapman et al., 2015). Current levels of coffee berry borer infestations average 15–20%, which causes yield loss, reduces quality and price, and increases costs (Aristizabal et al., 2016). If left uncontrolled, CBB can damage > 90% of coffee beans at harvest. Similarly, the tropical nut borer (TNB), *Hypothenemus obscurus* (F.) (Coleoptera: Curculionidae: Scolytinae), causes significant damage to macadamia (*Macadamia integrifolia* F. Muell.) nuts in Hawaii, especially if harvests are infrequent and the nuts are left uncollected after they fall to the ground (Jones 2002). Macadamia is grown in Hawaii on about 7300 ha and had an estimated farm gate value of $49 million in 2019–20 (USDA-NASS 2020, Pulakkatu-thodi et al. 2022). The estimated crop loss from TNB is 6–14 %, but it also introduces mold on the kernels, which may significantly increase losses (USDA-NASS 2020). Both CBB and TNB are cryptic because they complete their life cycles inside the berry or nut, making control difficult. In coffee, CBB control relies on well-timed application of the biopesticide *Beauveria bassiana* (Bals.-Criv.) Vuill., together with strip picking of coffee berries at the end of harvest (Aristozabal et al., 2016). No control measures are applied for TNB control in macadamia, but frequent harvests can limit damage.

Coffee berry borer and tropical nut borer arrived in Hawaii without their natural enemies. However, Jones (2002) reported that the cosmopolitan stored product pest *Cathartus quadricollis* (Guerin-Meneville) (squared-necked grain beetle) (Coleoptera: Silvanidae) could be found inside stick tight macadamia nuts (ones that do not abscise and remain on the tree) on the tree, and he showed that *C. quadricollis* could feed on TNB (Jones et al. 1998, Jones 2002). Soon after the arrival of CBB in Hawaii, *C. quadricollis* was discovered in coffee fields in infested berries feeding on eggs, larvae, and pupae of CBB (Sim et al. 2016, Follett et al. 2016). The *C. quadricollis* adult is long-lived and a strong flier that enters the coffee berry or macadamia nut through a bored hole to feed on CBB or TNB life stages. This predator is widely distributed in the coffee and macadamia-growing areas in Hawaii. In coffee, *C. quadricollis* feeds on CBB mainly in overripe and dried coffee on the tree rather than in ripening berries, where the initial crop damage occurs. *C. quadricollis* is not susceptible to mortality from applications of *B. bassiana* (Follett et al. 2016). In macadamia, TNB infests mature nuts that have fallen to the ground and stick tight nuts on the tree. Macadamia has indeterminate flowering and so flowers, developing nuts, and mature nuts can all be found on trees during most of the year; thus *C. quadricollis* can be found in TNB-infested macadamia nuts year-round.

Pierce et al. (1988) identified an aggregation pheromone for *C. quadricollis* as (3R,6E)-7-methyl-6-nonen-3-yl-acetate and named it ‘quadrilure’. Quadrilure is attractive to both sexes. Just as Scolytine ambrosia beetles are attracted to volatiles produced by cultures of their fungal symbionts (Hulcr et al. 2011, Kuhns et al. 2014), *C. quadricollis* and other flat bark beetle stored grain pests also feed on fungi associated with grain and those fungal volatiles might also serve as attractants (Pierce et al. 1991a, Thomas 1993, Follett et al. 2016). Therefore, we inferred that trapping efficiency of aggregation pheromones for *C. quadricollis* might be improved by the addition of volatiles from such fungi (Pierce et al. 1988).

Here, we report efforts to use the aggregation pheromone of *C. quadricollis* to manipulate the movement of this predator in coffee fields and macadamia orchards, and we conceived the idea of a field-based breeding station in the process. Our idea was to attract adult *C. quadricollis* beetles to food placed in stations with a pheromone lure or other attractants. We assumed that beetles attracted to such stations would feed on the food offered, multiply, and then disperse back into the field, augmenting the natural population. Experiments were conducted on methods to optimize the properties of the lure and breeding station for *C. quadricollis* and demonstrate proof of concept.

2. Materials and methods

2.1. Experiment overview

A series of experiments were conducted to optimize the components of a breeding station and then test it in the field. Experiments examining the effects of (1) pheromone lures and other attractants, (2) dispenser types, and (3) trap colors were conducted in a large macadamia nut orchard (MacFarms of Hawaii, Captain Cook, HI; 19.14533, −155.8540) on the western side of the Big Island of Hawaii. *C. quadricollis* can be found in TNB-infested macadamia nuts year-round, making macadamia orchards ideal for test lures, dispensers, and trap types.

Sticky traps with lures were arranged in a randomized complete block design in the macadamia nut orchard with 8–10 replicates per treatment (hereafter referred to as the trapping grid). Traps were placed at 1.5 m height in trees with a minimum separation of five trees (30 m) between traps within rows (blocks) and four rows (20 m) between blocks to form a grid pattern. Each experiment was conducted for 1- or 2-weeks duration and at the end of each experiment the adults of *C. quadricollis* captured on the traps were counted.

After the pheromone, dispenser type, and trap color studies were completed in the macadamia farm described above, the field tests with breeding stations were conducted on coffee farms. The coffee farms were in Kealakekua (Greenwell Farms; 19.51080, −155.92180; Lions Gate Farm, 19.47230, −155.89912) and Captain Cook (‘Rodeo’ farm, 19.45595, −155.89226), Hawaii and varied in elevation from 400 to 450 m. Coffee is a 7-month crop from flowering to harvest and coffee berries of different maturity stages can be found on the tree during the season due to multiple flowerings. *C. quadricollis* can be collected on farms throughout the season but is especially abundant later in the season when overripe and dried coffee berries that are heavily infested with CBB are available (Follett et al. 2016, Brill et al.2021).

Quadrilure pheromone lures (loaded with 1 mg of 2:3 blend of (R)-(**E**)-7-methyl-6-nonen-3-yl acetate and (R)-(Z)-7-methyl-6-nonen-3-yl acetate) and the release dispensers used in experiments were supplied by Alpha Scents, Inc. (Canby, Oregon). Preliminary tests showed that lures loaded with 1 mg quadrilure pheromone caught significantly more *C. quadricollis* than lures loaded with 0.1 or 0.01 mg quadrilure, and so the 1 mg rate was used in all tests presented here. In some experiments a fungal odor blend lure was used in addition to quadrilure to attract *C. quadricollis*. The fungal odor blend, which included ethyl acetate (1.88 g), ethanol (1.5 g), 3-methyl-1-butanol (1.12 g), and isoamyl acetate (0.625 g) (36.5:29:22:12.5 mixture by volume) (Kuhns et al. 2014), was presented in a black semipermeable polyethylene pouch.

2.2. Dispenser type and trap color

We evaluated the effect of different types of quadrilure dispensers on

C. quadricollis captures. Using yellow double-sided sticky card traps (19 × 14 cm, Alpha Scents, Canby, OR) arranged in the replicated grid design, different dispenser types with quadrilure were tested in combination with the fungal volatiles lure. Lures were attached with staples or twist ties to the top of the yellow sticky cards. Five different quadrilure dispensers were compared: (1) 1 mg of quadrilure in an open 250 µL polyethylene vial and a fungal volatiles pouch (called 'vial'), (2) 1 mg of quadrilure mixed inside the fungal volatiles pouch ('phero-inside'), (3) 1 mg of quadrilure loaded on a red rubber septum and a fungal volatiles pouch ('red septa'), (4) 1 mg of quadrilure loaded on a gray rubber septum and a fungal volatiles pouch ('gray septa'), and (5) 1 mg of quadrilure loaded on a membrane-type wafer and a fungal volatiles pouch ('wafer'). Yellow sticky card traps with no lures were included in the test as controls. Captured insects were counted after one week.

In another experiment, double-sided sticky card traps (19 × 14 cm, Alpha Scents, Canby, OR) of various colors were compared for their attractiveness to *C. quadricollis* using the combination of the quadrilure membrane-type wafer lure and fungal volatiles lure. Seven sticky card colors—red, green, black, blue, red, white, and clear—baited with the quadrilure pheromone lure and the fungal volatiles lure were deployed in the replicated trapping grid. Yellow sticky card traps with no lures were included in the test as controls. Captured insects were counted after one week.

2.3. Lure release rate

The quadrilure membrane-type (wafer) captured the most *C. quadricollis* adults in the dispenser-type comparison experiment and so the wafer was used in all subsequent experiments. The wafer lure was studied in the laboratory to determine the rate of quadrilure release over the course of eight weeks. Lures were aged in a fume hood at 17 °C, which is typical of early morning and nighttime temperatures when *C. quadricollis* adults are most active (Acebes-Doria et al., in review). The headspace volatiles of the wafer lure were collected using 6L closed volatile collection chambers (Chemglass, Vineland, NJ, USA) with one quadrilure membrane lure loaded with 1 mg quadrilure per chamber, as described in Cha et al. (2012). The chambers had one air inlet adapter (7 mm ID) on the top and an outlet adapter (7 mm ID) on the bottom wall. Air was pulled through an activated charcoal trap (ORBO32-small, Supelco Inc., Bellefonte, PA, USA) into the chamber at 1 L min^{-1} through the top inlet port, and headspace volatiles from the lure was pulled through an adsorbent trap (50 mg HayeSep-Q) at the bottom of the chamber. The level of volatiles trapped was sampled every 8 h for the first 24 h, then every day for the next six days, then three times per week for the next three weeks, and then once per week for four weeks, for a total sampling time of eight weeks. Each headspace collection lasted for three hours. Collected volatiles were extracted from the adsorbent trap with 1 mL of dichloromethane. The extracts were kept at − 20 °C until subjected to gas chromatography-mass spectrometry (GC–MS) analysis.

Samples were analyzed using an Agilent 7890B GC coupled to an Agilent 5977A mass selective detector (MSD). The GC was fitted with a HP-5MS column (30 m × 0.25 mm ID × 0.25-µm film; Hewlett-Packard), which was programmed at 40 °C for 5 min, then increased at 5 °C/min to 150 °C. The injector and transfer line temperatures were set to 280 °C, and injections were made in splitless mode with 1 min of sampling. The first week's samples were analyzed in full scan mode with electron impact ionization, and thereafter it was necessary to analyze the samples with single-ion mode (SIM) due to the low concentration. The SIM method was set to scan for *m/z* ions 67.1, 109.1, 138.2, all diagnostic for quadrilure. Quadrilure levels from the adsorbent collections were quantified based on total ion abundances (5 to 250 ng/µL) or selected single ion abundances (0.005 to 50 ng/µL) from GC–MS analyses according to standard curves made from neat quadrilure.

2.4. Breeding station design

A prototype breeding station was designed that would attract live *C. quadricollis* adults to a food source suitable for their reproduction and larval development. The station consisted of a 1.57 L juice bottle with two screened sides (9 × 10 cm, window screen) to allow entry of *C. quadricollis* and a roof to keep rain out of the bottle (Fig. 1). The bottom of the bottle was filled with 250 g of food (cracked corn and cornmeal at 4:1, w:w) and a lure was placed on top of the food to attract *C. quadricollis* into the station through the screen and onto the food.

2.5. Laboratory test with breeding stations in buckets

We conducted a laboratory experiment to determine how many *C. quadricollis* might be produced in a breeding station (as described above) in containment using buckets after stocking the breeding stations with a known number of adult *C. quadricollis*. We used modified plastic, 18.9-liter 'paint' buckets (33 cm diameter and 36 cm height) in which we inserted breeding stations, which were then observed to detect *C. quadricollis* reproduction and dispersal in confinement. A 13 cm diameter ventilation hole was cut in the center of each bucket lid and the hole then covered with fine mesh, attached with hot glue. A 0.5 × 2 × 22 cm wooden stick was hot glued to the underside of the lid so that it crossed over the center of the bucket. A breeding station could be suspended from the center of the wooden stick attached to the bucket lid using a thin galvanized wire. When the breeding station was suspended as described it did not touch the sides or bottom of the bucket. Inside each bucket, the bottom and side walls were lined with removable clear plastic sticky panels (Alpha Scents, West Linn, OR) to capture any adult *C. quadricollis* leaving (dispersing) from the stations.

Twelve breeding stations were used that contained either 1) 150 g food (five replicates) or 2) 250 g food (five replicates) food, or 3) an equal volume of paper shavings as a control (two replicates). One hundred *C. quadricollis* adults were taken from a laboratory colony maintained at the USDA-ARS, U.S. Pacific Basin Agricultural Research Center (Hilo, Hawaii) and added to each breeding station, which were then suspended individually inside buckets. At weekly intervals for five months, buckets were opened, and the sticky paper was removed to

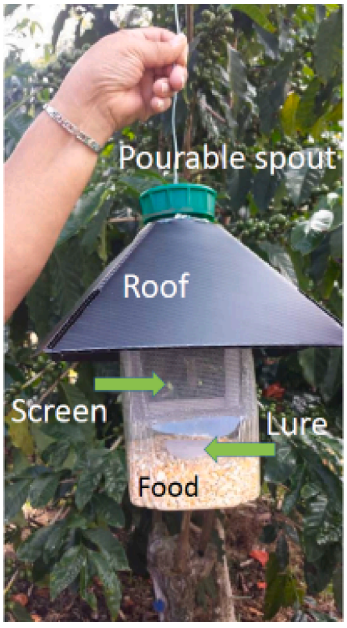

Fig. 1. The breeding station components. *Cathartus quadricollis* adults are attracted to the aggregation pheromone (quadrilure), and pass through the screen into the station and find the food. The food and lure are inserted through the spout, and the roof helps keep the food dry.

count *C. quadricollis* that had dispersed from the breeding stations. Each week, buckets were relined with fresh sticky paper.

2.6. Field test of breeding stations using buckets

In the field experiment, the breeding stations became infested with wild *C. quadricollis* that were naturally attracted to the lures in the stations. Commercially available *C. quadricollis* breeding stations (Alpha Scents, Camby, OR) were deployed in a coffee field and the breeding stations were baited with either two pheromone lures or a pheromone lure and a fungal lure. All breeding stations also contained 250 g food (cracked corn/cornmeal). These breeding stations were placed at five locations on each of two coffee farms ('Rodeo' farm, Greenwell Farms) and each station was spaced 30 m apart from any others within the farm. The stations were hung approximately 1 m off the ground on wooden posts driven into the ground between rows. A sticky barrier (Tanglefoot, Grand Rapids, MI, USA) was applied to the base of the posts to prevent access to the breeding stations by ants. After five weeks, all breeding stations were brought back to the laboratory and set up in plastic buckets (as described above) lined with sticky panels and held for five months to allow *C. quadricollis* to multiply. At weekly intervals, buckets were opened, and sticky panels removed to count *C. quadricollis* adults and any other beetles dispersing from the stations.

2.7. Field weathering test

An experiment was conducted in the field to determine how the attractiveness of breeding stations baited with one pheromone lure each changed over time. A replicated study was conducted at Lions Gate Farms (Kealakekua, HI) with four blocks. Blocks were separated by approximately 100 m, and stations within blocks were separated by approximately 30 m. Each block contained one replicate with each of four treatments, which were breeding stations with a pheromone lure that had been weathered for either (1) 0 days (newly opened), (2) 7 days, (3) 14 days, or (4) food only (the "no lure" controls). The pheromone lures were weathered by placement outdoors in the shade at 14 or 7 days before the start of the experiment. Each breeding station was suspended at 1.5 m above the ground from a 2.5 cm-diameter fiberglass pole positioned between two coffee plants. Stations were retrieved after one week and adult *C. quadricollis* in the food were counted. The exact time of entry of *C. quadricollis* into the breeding station could not be known exactly, and lures would have continued to weather during the experiment. Therefore, treatments (in terms of age of pheromone lures) would be 0–6 days, 7–13 days, and 14–20 days.

2.8. Statistical analysis

All trap and breeding station count data were evaluated for normality, then subjected to analysis of variance (ANOVA) using JMP v. 16 statistical analysis software (SAS, 2021). In some cases, data were log-transformed after examining the data's distribution if this improved normality. For significant effects, mean separations were done using a *t*-test or Tukey's HSD test.

3. Results

3.1. Release device and trap color

In the experiment comparing pheromone dispenser types in combination with black pouches containing a fungal volatiles blend, the effect of dispenser type was significant ($F = 6.2$; df = 4, 28, $p = 0.001$), but all dispensers were effective in attracting *C. quadricollis* and could potentially be used to attract founders to a breeding station. The wafer + fungal blend treatment caught the most insects (Fig. 2). Black sticky

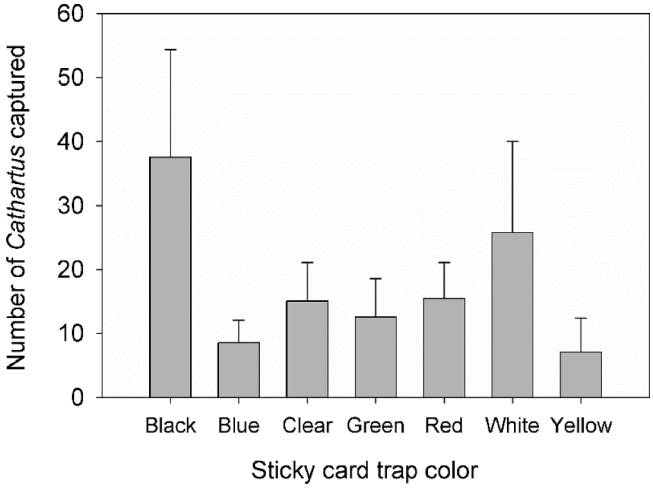

Fig. 3. Captures of *Cathartus quadricollis* on sticky traps of different colors using a quadrilure lure and a fungal volatiles lure together as attractants.

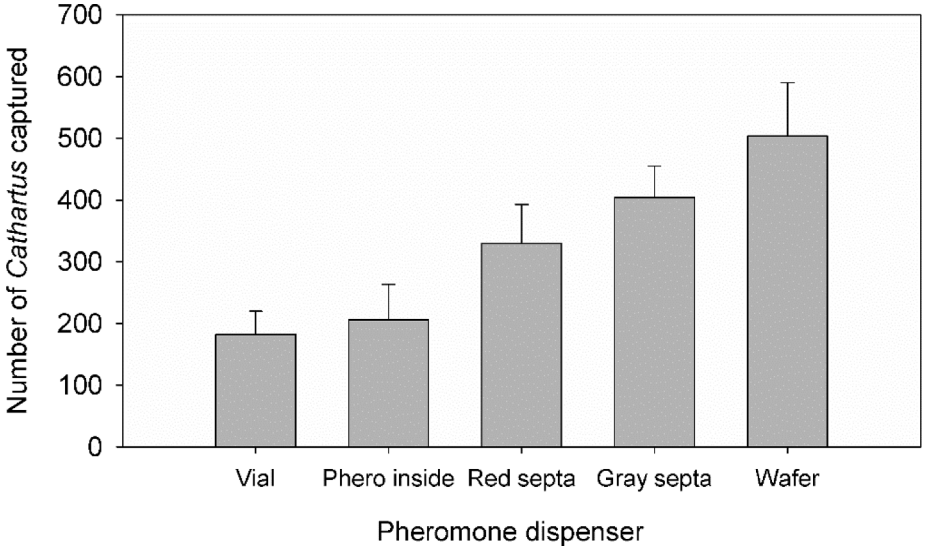

Fig. 2. Captures of *Cathartus quadricollis* on yellow sticky traps using different dispensers for quadrilure (1 mg).

traps caught the most *C. quadricollis,* but the effect of trap color was variable and not significant (F = 1.1; df = 6, 63; $p = 0.09$) (Fig. 3). In both experiments, yellow sticky traps with no lures serving as controls caught no insects.

3.2. Pheromone + fungal lure vs. pheromone only

Yellow sticky traps baited with the quadrilure lure plus the fungal volatiles lure caught significantly more *C. quadricollis* than the quadrilure lure alone (t = 6.7, df = 1,18, $p = 0.012$) (Fig. 4). The quadrilure alone caught only *C. quadricollis* (82.9 ± 37.1 SE), but the quadrilure + fungal volatiles lure caught *C. quadricollis* (242.2 ± 49.1) and several other insects including *Carpophilus* sp. (Coleoptera: Nitidulidae) (9.5 ± 2.7), *H. obscurus* (tropical nut borer) (4.1 ± 1.2), *Xylosandrus compactus* (Eichoff) (Coleoptera: Curculionidae: Scolytinae) (black twig borer) (1.4 ± 0.6), and the fruit flies *Bactrocera dorsalis* (Hendel) (Oriental fruit fly) (34.7 ± 4.0, 4:1 male:female), and *Ceratitis capitata* (Wiedemann) (Mediterranean fruit fly) (2.5 ± 0.8) (Diptera: Tephritidae). The yellow sticky traps with no lures (controls) caught no insects.

3.3. Lure release rate

The initial release of quadrilure from the membrane-type (wafer) lures was 11,119 ± 703 ng/h (mean ± SE), which quickly dropped off to 2,208 ± 330 ng/h after the first eight hours of release (Fig. 5a). The release rate dropped further in first few days to 73.7 ± 7.8 ng/h by day 4, and then to 13.5 ± 0.5 ng/h by day 7 (Fig. 5b). By day 16, the release rate had fallen to about 5 ng/h and remained at that rate through day 57, which was likely to elicit minimal attraction to *C. quadricollis*. These quadrilure lure release rates were considerably lower than those reported for codling moth *Cydia pomonella* (L.) at the same loading rate (1 mg/lure) (Liu et al. 2016) but sufficient to outcompete adult *C. quadricollis* in the field and attract individuals to the breeding station (see 3.6, *Field Weathering Test*).

3.4. Laboratory test with breeding stations in buckets

The predator breeding station (Fig. 1) was stocked in the laboratory test with 100 adult *C. quadricollis* and with one of two levels of food. The higher food level (250 g) treatment yielded significantly more *C. quadricollis* adults than the lower food level (150 g) treatment (t = 12.1, $p < 0.0001$) (Fig. 6). Approximately 30 of the original 100 *C. quadricollis* per station dispersed from the breeding stations in the first week of the experiment (before the production of any offspring), then catch of dispersing adults was very low for about two months. At 8 weeks, large numbers of *C. quadricollis* began dispersing from the stations in both the 250 g (mean = 711.2 captures) and 150 g (mean = 722.8 captures) treatments, and *C. quadricollis* continued to disperse from stations for the remainder of the experiment (Fig. 6). Peak capture in the 250 g food treatment was a mean of 1540.2 adults on week 15 (Fig. 6). In total, the original 500 *C. quadricolis* adults (100 per station, 5 stations) produced 25,992 and 51,184 *C. quadricollis* in the 150 g and 250 g treatments, respectively, during the four months of the experiment (an estimated 3–4 overlapping generations), for an average of about 5,000 and 10,000 per station for the low and high food levels.

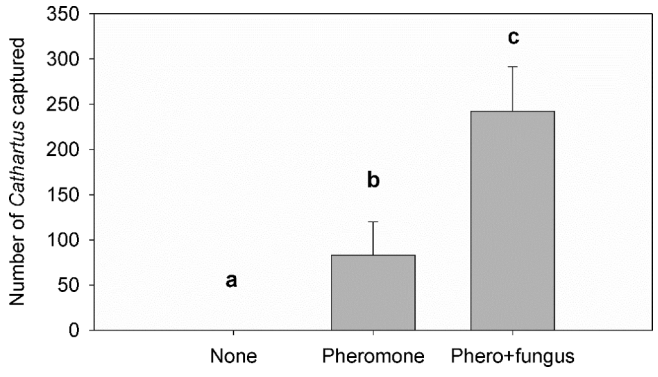

Fig. 4. Comparison of yellow sticky trap captures of *Cathartus quadricollis* when using quadrilure (Phero) alone or quadrilure and a fungal volatiles lure together (Phero/Fungus). Bars with different letters are significantly different by Tukey's test.

3.5. Field test of breeding stations using buckets

In the next experiment, breeding stations were first hung on stakes on a coffee farm for five weeks for natural infestation by *C. quadricollis* and then returned to the laboratory where they were placed in buckets to follow and quantify beetle emergence. Over 19 weeks while being held in buckets, breeding stations produced an average of 3028.8 ± 389.4 *C. quadricollis* adults (n = 5, range 2080–3959) when the pheromone/fungus lure was used and an average of 2959 ± 787.1 *C. quadricollis* adults (n = 5, range 1013–5178) when the pheromone alone was used, which numbers were not significantly different (t = 1.25, df = 1, 188; $p = 0.27$) (Fig. 7). For bycatch, *Carpophilus* sp. adults also emerged from the breeding stations in a pattern spread over 19 weeks, suggesting that they reproduced in the breeding stations; yielding 286 *Carpophilus* adults from breeding stations with pheromone + fungal volatiles (n = 5 stations) versus 240 from stations with only pheromone lures (n = 5 stations). Whether they were feeding on the corn food or attacking *C. quadricollis* life stages is unknown. A small number (<20) of *Leptophloeus* sp. (Coleoptera: Laemophloeidae) adults were also recovered from the breeding stations; this genus is another group of flat bark beetle predators known to feed on CBB (Follett et al. 2016, Brill et al. 2021).

3.6. Field weathering test

There was a strong decline in lure attraction for lures weathered (before the experiment) for different numbers of days before field exposure. The mean number of *C. quadricollis* attracted over one week to breeding stations deployed in coffee was 28.8 (SE ± 8.0), 5.3 (SE ± 3.3), and 0.5 (SE ± 0.5) for stations with lures weathered for 0, 7, or 14 days (F = 21.3, df = 3, 15; $p < 0.0001$). No *C. quadricollis* were recovered from the food-only control. This shows that most of the first-generation *C. quadricollis* arrive soon after placement of the breeding station in the field. This result is consistent with the lure release rate measurements presented above (see 3.3. *Lure Release Rate*) that showed high initial release rates soon decreased dramatically (Fig. 4).

4. Discussion

Our laboratory and field studies identified the optimal quadrilure dispenser type (membrane) and trap color (black) for attracting *C. quadricollis*. A predator breeding station consisting of a screened and sheltered enclosure containing food (250 g, a cracked corn, cornmeal mixture [4:1]) and a membrane-type pheromone lure (with 1 mg quadrilure) was developed to augment predator numbers in coffee and macadamia fields. In the laboratory, the breeding station was shown to be capable of increasing *C. quadricollis* numbers 100-fold over a four-month period. In field tests in coffee, quadrilure was shown to attract wild adults to breeding stations, where they multiplied and later dispersed from the stations. Combining two lures (quadrilure and fungal volatiles) increased the numbers of *C. quadricollis* attracted to breeding stations but also caused some bycatch of non-target species. Because of the bycatch effect when adding fungal volatiles to the lure and the unknown effects of having multiple species reproduce in the food station, use of the quadrilure lure alone is recommended. Attracting more *C. quadricollis* to the breeding station might accelerate multiplication and dispersal, but *C. quadricollis* is long-lived and even a small number of

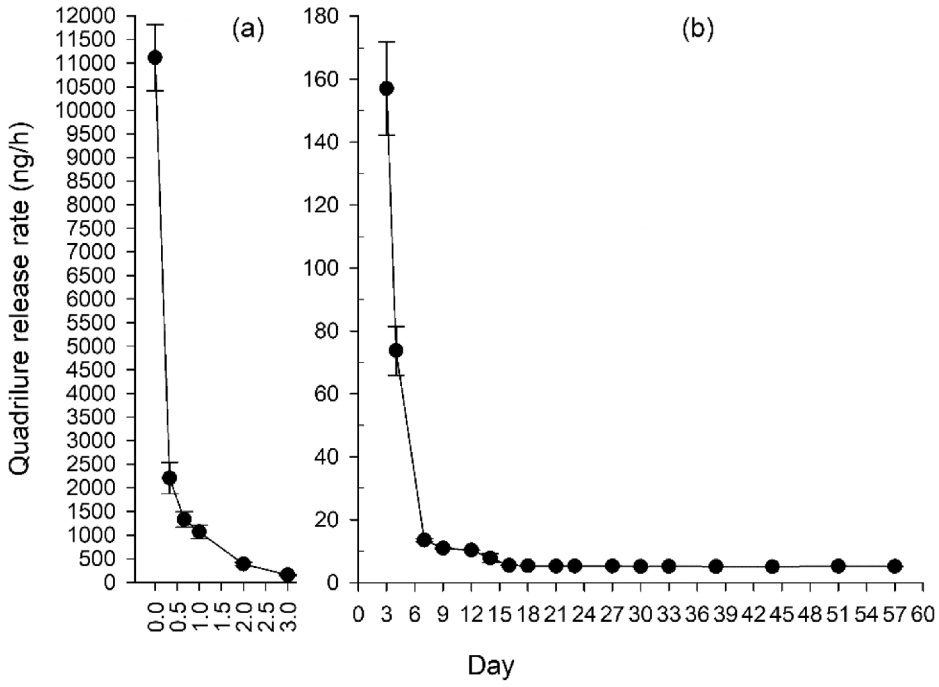

Fig. 5. Mean (±SE) nanograms per hour of quadrilure released from an Alpha Scents membrane-type lure (wafer), (a) days 0 to 3, (b) days 3 to 57.

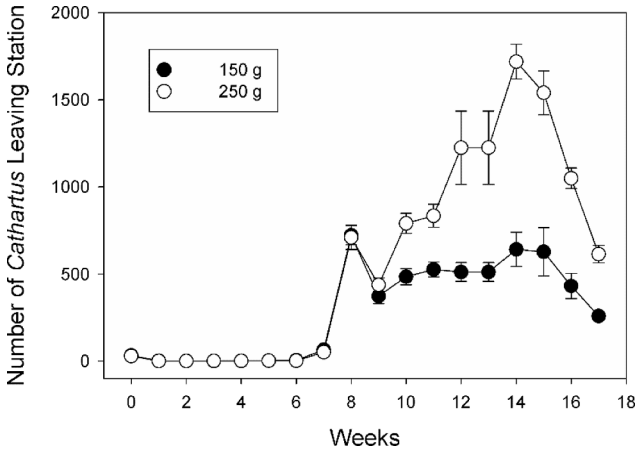

Fig. 6. Weekly numbers (mean ± SE) of *Cathartus quadricollis* dispersing from breeding stations in buckets in the laboratory after stocking with 100 individuals (mixed males and females) from a laboratory colony. Stations had either 150 g or 250 g of corn/cornmeal food. Numbers dispersing were significantly higher in the 250 g versus 150 g treatment (*t*-test, $p < 0.0001$).

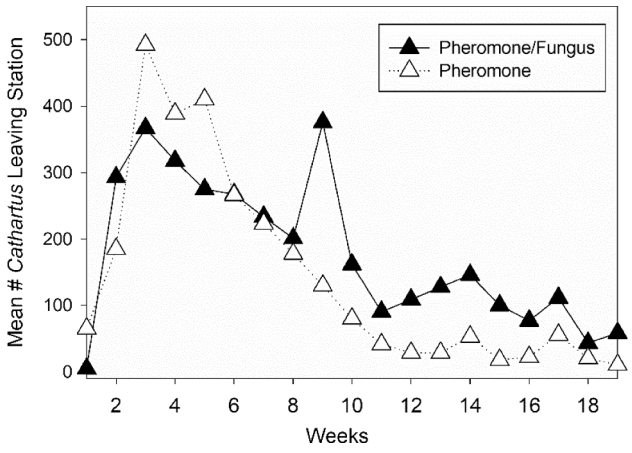

Fig. 7. Weekly mean numbers of *Cathartus quadricollis* dispersing from breeding stations in buckets in the laboratory over 18 weeks after placement in a coffee field for five weeks for attraction of wild individuals using a quadrilure pheromone lure alone (Pheromone) or a quadrilure lure and a fungal volatiles lure together (Pheromone/Fungus). Numbers dispersing from breeding stations with different lures were not significantly different (*t*-test, $p = 0.27$).

founders can produce high numbers of dispersing individuals during a season during multiple overlapping generations. Breeding stations were an efficient way to multiply the predator *C. quadricollis* in the field and could be used to augment biological control of the coffee berry borer in coffee and tropical nut borer in macadamia nut.

Quantifying any increased mortality caused by augmented numbers of *C. quadricollis* in the crop would be difficult because both pests of interest (CBB and TNB) are cryptic, being found inside the coffee berry or macadamia nut. In laboratory bioassays, Follett et al. (2016) showed that *C. quadricollis* adults can feed on CBB eggs, larvae, and pupae. Laiton et al. (2018) showed that *C. quadricollis* adults and larvae will feed on CBB in infested coffee berries. The use of artificial coffee berries with sentinal prey that can be easily counted before and after placement in the field is a technique that can be used to quantify predation in the field. Brill et al. (2021) found that 40% of the eggs placed inside artificial berries on coffee plants were consumed by *C. quadricollis*. Liang et al. (2023) showed that *C. quadricollis* will feed on all CBB life stages in artificial coffee berries placed in coffee fields, with predation rates on different life stages ranging from 12 to 49%. Importantly, Brill et al. (2021) showed that laboratory-reared *C. quadricollis* were recaptured near release sites on coffee farms at 1, 2, and 7 weeks after augmentative releases, indicating that these predators will remain in fields if prey are abundant. Future studies are needed to link increased numbers of predators with reduced crop damage.

C. quadricollis can also be found in many wild plants with seed pods, which may help allow the predator to persist when pest densities are low. In recent field studies in Hawaii, all life stages of *C. quadricollis* were found in the seed pods of *Leucaena leucocephala* (Lam.) de Wit and several other leguminous trees located near coffee or macadamia farms,

presumably feeding on non-pest scolytines. Feeding on non-pest scolytines in such non-crop plants may help this predator persist in coffee and macadamia plantations (Brill et al., 2021). Feeding on multiple pests in multiple crops will also favor persistence. Molecular marker studies showed that *C. quadricollis* feeds on the coffee and macadamia pest species CBB and TNB, as well as on the more polyphagous pest species black twig borer, *X. compactus*, which is a pest of coffee and many other plants in Hawaii (Brill et al., 2021).

Flat bark beetle predators such as *C. quadricollis* have potential for augmentative biological control. In coffee, *C. quadricollis* mainly attacks CBB in dried coffee berries left on the tree after harvest (Follett et al. 2016). Therefore, its role in CBB management will be to suppress population growth in unharvested coffee between seasons and in abandoned coffee. Many Hawaii coffee growers have been given predator 'starter kits' containing *C. quadricollis* and food (corn and cornmeal) in a plastic bucket and are periodically releasing home-grown predatory beetles on their farms to augment existing populations (Follett et al. 2016). Currently, farmers are supplied and often re-supplied with 'starter' beetles raised in our laboratory. A system for farmers to sustainably rear *C. quadricollis* on-farm using breeding stations containing *C. quadricollis* pheromone lures and food might facilitate wider grower adoption of the predator augmentation program. The simplicity of do-it-yourself construction of a predator breeding station and the commercial availability of the lures and breeding stations at Alpha Scents, Inc. (Canby, OR) make use practical.

When a new biological control agent begins to be used in a crop, its use must be compatible with other current management practices. In the case of CBB, this species spends most of its life cycle inside coffee berries. The adult females bore into berries, and each female lays 35–50 eggs, which have a highly female skewed sex ratio (13:1, F:M) (Baker et al. 1992). The lifespan for females is 35–190 days and for males, 40 days. Mating occurs inside the berry, most often between siblings. Some females lay eggs in the same coffee plant, others colonize new ones, but the males never leave the berry. The same plant can host three to five generations of beetles. The cryptic nature of CBB feeding and reproduction inside the berry makes it difficult to control with chemical pesticides. The predator we studied, *C. quadricollis*, readily enters infested coffee berries, where it feeds on immature CBB life stages (eggs, larvae, pupae). Currently pest management practices for CBB in coffee rely on application of a biopesticide based on *B. bassiana* and sanitation (stripping of remaining coffee berries after harvest) (Aristizabal et al. 2016). Using a laboratory bioassay, Follett et al. (2016) showed that *C. quadricollis* is not susceptible to *B. bassiana*, so the adult predators should not be affected by current use of this insecticide in the field. Strip picking and removal of unused coffee berries will reduce the availability of prey, but *C. quadricollis* has shown resilience and persistence even in carefully managed coffee farms that employ strip picking (Follett et al. 2016).

Generalist predators of insect pests that have been most studied in crop IPM programs have been lady beetles (Coleoptera: Coccinellidae), hover flies (Diptera: Syrphidae), lacewings (Neuroptera: Chrysopidae, Hemerobiidae), ground beetles (Coleoptera: Carabidae), and various Hemiptera (e.g., Anthocoridae, Nabidae, Pentatomidae) (Hagen et al. 1999). In contrast, flat bark beetles (e.g., Silvanidae and other families) are an understudied group of predators that have potential for augmentative biological control, particularly against scolytine crop pests (Thomas 1993). Luring carnivorous insects into crops using flowers, plant volatile compounds, or pheromones can increase their numbers and provide increased pest mortality (Kaplan, 2021). But attracting predators into a crop means drawing them away from a source area where they may be providing biological control in a crop or ecosystem services in a natural habitat. Predator breeding stations circumvent this source-sink problem by propagating predators in the crop of interest. *C. quadricollis* is unusual in that it is an omnivore that feeds on scolytine prey without feeding on the crop. Its dual lifestyle as a stored grain pest allows propagation in the field in breeding stations to augment populations, thereby potentially increasing encounters with and control of the crop pest. Other flat bark beetles may also be both stored grain pests and predators (e.g., *Cryptolestes* spp., *Oryzaephilus* spp.), and some may have pheromones or other attractants that have been identified (Phillips 1997), and therefore amenable to augmentation using breeding stations. In Hawaii, the flat bark beetle predator *Leptophloeus* sp. (Coleoptera: Laemophloeidae) has been found in coffee berries feeding on CBB (Follett et al., 2016) and in macadamia nuts feeding on tropical nut borer (Brill et al., 2021), and like *C. quadricollis* it can be reared on corn and other grains. The foreign grain beetle, *Ahasverus (*formerly *Cathartus) advena* (Waltl) (Coleoptera: Silvanidae) feeds on the mold growing on grains, peanuts, and dried fruits, but it has also been found feeding on coffee berry borer in coffee in Colombia (Laiton et al. 2018). 1-Octen-3-ol is an attractive semiochemical for *A. advena* (Pierce et al. 1991b), which could be used as an attractant in a breeding station.

5. Conclusions

The ecology of flat bark beetles is poorly understood (Thomas 1993), and their role as insect predators deserves greater attention, particularly for scolytine pests. In Hawaii, the predator *C. quadricollis* was found naturally attacking coffee berry borer in coffee berries and tropical nut borer in macadamia nuts. *C. quadricollis* is also a cosmopolitan stored product pest that is easily reared in stored grains. A predator-breeding station was conceived as an efficient way to augment *C. quadricollis* numbers in the field. A pheromone was used to attract *C. quadricollis* to the breeding station where they fed and multiplied on the food provided, then dispersed naturally back into the field. We demonstrated that large numbers of predators can be produced in the breeding station and around it but did not attempt to measure the effects of augmented predator numbers on pest density or crop damage. Future studies will attempt to quantify increased predation rates on pests in the presence of predator breeding stations. Other flat bark beetle species may be both predators and stored grain pests and therefore suitable for augmentative biological control using breeding stations.

CRediT authorship contribution statement

Peter A. Follett: Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Darek Czokajlo:** Methodology, Writing – review & editing. **R. Max Collignon:** Methodology, Data curation. **Dong Cha:** Methodology, Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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