

## Estimating the effective sampling area of an alcohol-baited trap for monitoring the coffee berry borer

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**ABSTRACT:** Here, single-trap, multiple-release-recapture field experiments were used for the first time to estimate the trap sampling area and absolute population density from trap catches for the coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari). Fluorescent-dusted CBBs were released at several distances at all four cardinal points from an ethanol-methanol-baited multifunnel trap. Only 2.6 % of released beetles were recaptured, and recaptures decreased significantly with increasing release distances. The recapture analyses revealed that CBB moves randomly in the field and disperses at a maximum of 22.2 m. Despite the short plume of the trap (1.3 m), the calculated trap sampling area was 0.17 ha, with an overall catch probability of 0.01. Therefore, capturing 100 CBBs trap<sup>-1</sup> ha<sup>-1</sup> at the early filling stage of coffee beans reflects a 20.2 million borers ha<sup>-1</sup> population at harvest. This results in a projected bean loss of 60.3 kg ha<sup>-1</sup>. The findings shed light on improvements in using semiochemical traps and interpretations of catch data to enhance the CBB integrated management. Future studies on the performance of attractants and trap designs in different coffee farmscapes are encouraged to adopt the present methodology.

**Keywords:** IPM, Scolytinae, marked insects, fluorescent dust, semiochemicals

### Introduction

Semiochemical-baited traps are pivotal in integrated pest management (IPM) in agricultural and forestry systems (Gut et al., 2004; Witzgall et al., 2010); nevertheless, translating trap catches accurately into absolute pest density, a crucial step in determining economic thresholds has proven challenging (Miller et al., 2015; Miller, 2020). Recent research has concentrated on refining methods to estimate the effective sampling area of these traps, utilizing theoretical, computational, and field-based approaches (Adams et al., 2017a, b; Miller et al., 2015; Kirkpatrick et al., 2018, 2019; Onufrieva et al., 2020; Onufrieva and Onufriev, 2021).

This critical parameter defines the area within which insects can reach a trap and has been investigated through single-trap, multiple-release-recapture experiments involving fluorescent-dusted insects (Turchin and Odendaal, 1996; Miller et al., 2015). Determining this sampling area is essential to calculate trap density, estimate absolute pest population density, and predict crop damage, thereby guiding pest management tactics (Miller et al., 2015).

For the coffee berry borer (CBB), a devastating coffee pest, alcohol-baited traps have been the primary means of capture due to the beetle life cycle inside the coffee berry (Vega et al., 2015). Effective management requires targeting dispersing beetles before berry colonization, making semiochemical-baited traps crucial to detect their field movements (Vega et al., 2015).

Despite extensive research on the use of these traps for CBB monitoring, consensus remains elusive regarding trap spacing and catch data interpretation, limiting the precision of IPM strategies (Mathieu et

al., 1997b; Mathieu et al., 1999; Fernandes et al., 2011; Messing, 2012; Pereira et al., 2012; Aristizábal et al., 2017; Johnson et al., 2018; Oliveira et al., 2018; Souza et al., 2020; Johnson and Manoukis, 2020, 2021; Ruiz-Diaz and Rodrigues, 2021).

This study pioneers the application of single-trap, multiple-release-recapture experiments to define the sampling area of an ethanol-methanol-baited trap for CBB monitoring and estimate its population density. Our specific objectives were to: 1) quantify the trap plume reach and maximum dispersal distance of the CBB; 2) estimate the trap sampling area and overall catch probability within the trapping area; 3) translate the number of trapped CBBs into the absolute population density; and 4) calculate the damage to coffee production by the pest.

### Materials and Methods

#### Test site

Field bioassays were conducted in the center of a 3-ha field of Arabica coffee (*Coffea arabica* L., var. Catuaí Vermelho IAC-44) on a Santa Teresa Farm, Espírito Santo, Brazil (19°55'44.3" S, 40°44'38.1" W, altitude 450 m). The coffee trees were approximately 2 m high, three years old, and grown at full sun at a spacing of 2.5 m (between rows) × 1 m (within rows), with a density of approx. 4000 trees ha<sup>-1</sup>. The plot was neighbored by areas of Atlantic Forest remnants (North and East faces), eucalypt (South), and Arabica coffee (same variety above; West). The experimental plot received no biological or chemical control for CBBs for one year before and during the tests.

## Source of beetles

Adults of CBB used for the release-recapture experiments were reared from infested coffee berries at the "raisin" stage collected from an Arabica coffee field adjacent to the abovementioned field on 14 May 2019. Coffee berries were disinfected with a solution of water, dish detergent, and sodium hypochlorite, according to the protocol of Silva et al. (2012), to minimize fungal contaminants. Next, a single layer of treated berries was placed in plastic containers lined with paper towels and covered with white voile fabric. The containers were kept in a shed (approx. 25 °C, 60 % relative humidity, and approx. L12:D12 photoperiod) on the farm. The containers were placed on a windowsill under indirect sunlight to stimulate beetle emergence (Mota et al., 2017). Containers were checked hourly for emerging beetles from 13h00 to 17h00, corresponding to the peak interval of colonizing females leaving the berries (Baker et al., 1992; Mathieu et al., 1997a). The age of the beetles could not be estimated as they emerged from field-infested coffee berries. However, only CBBs up to 2 d after emergence (individualized in empty containers), which displayed a high capacity for flight and movement, were used for the assays.

## Marking CBBs

In the morning of the tests (9h00), emerged CBBs were placed in groups of 100 individuals into 45-mL centrifuge tubes and then transferred to Petri dishes (90 mm diameter × 15 mm high) lined with filter paper containing roughly 4 mg of DayGLO fluorescent dust (DayGLO Color Corporation). The dishes were kept under LED light for 2 h to stimulate the insects to move, improving the coverage of their bodies with fluorescent dust. This protocol has been successfully validated for marking CBB (Acevedo-Bedoya et al., 2009) and several other scolytine species (Turchin and Odendaal, 1996; Byers, 1999; Poland et al., 2000; Dodds and Ross, 2002; Doležal et al., 2016; Meurisse and Pawson, 2017), where the fluorescent dust has not affected longevity, flight initiation, and semiochemical detection of the marked beetles. Groups of 100 CBBs for each release distance were dusted with a different DayGLO fluorescent color, including ECO11 Aurora Pink®, ECO13 Rocket Red™, ECO15 Blaze Orange™, ECO17 Saturn Yellow®, ECO18 Signal Green™, ECO19 Horizon Blue™, and ECO21 Corona Magenta™. The marked insects were transferred to a Petri dish containing only filter paper and transported to the field inside a black plastic container to prevent light stimulation.

## Semiochemical-baited trap

We used a custom-made red 4-unit funnel trap modeled after the traps used by Lindgren (Lindgren, 1983; Mathieu et al., 1997b) (Figure 1A). The trap basin was fitted with a collection jar (7 cm diam. × 14 cm high)

lined with sticky black cardboard at the bottom to retain and preserve the marked CBBs. The attractant consisted of a 50-mL polyethylene flask filled with a 1:1 mixture of 99 % ethanol and 99 % methanol. The flask was fitted with a 70-mm-long cotton-string wick inside a polyethylene pipe (40 mm long × 2 mm diameter) to provide a constant and uniform release of the alcohol mixture at approximately 1 g d<sup>-1</sup>, which was determined by gravimetric release tests following the methods by Sullivan (2005) (data not shown). The proportion and release rate of the alcohols adopted here have been proven optimal for CBB attraction (Mathieu et al., 1997b; Mathieu et al., 1999; Silva et al., 2006; Dufour and Frérot, 2008). The attractant was hung in the trap center, and the trap was suspended on a 1.5 m inverted-L steel reinforced bar fixed to the ground, with the dispenser at roughly 1.2 m above the ground.

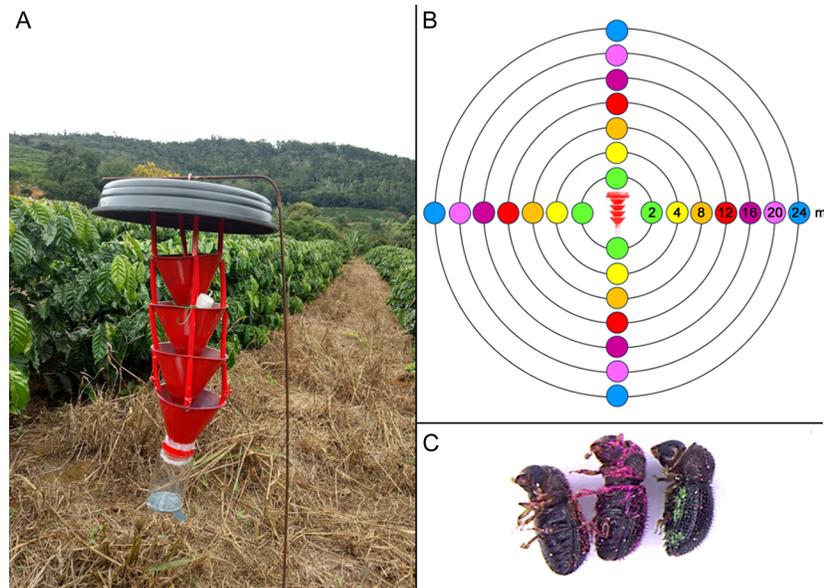
## Release-recapture of marked CBBs

Groups of 100 females of CBBs were released in the four cardinal points at 2, 4, 8, 12, 16, 20, and 24 m from a central alcohol-baited trap (Figure 1B), totaling 400 beetles dusted with the same fluorescent color per distance. Beetles were released at 15h00, that is, during the daily flight activity peak of CBBs (Baker et al., 1992; Mathieu et al., 1997a), by opening the Petri dishes on brick platforms 0.5 m above the ground and allowing the beetles to fly freely. The trap was serviced once a day at 18h00 (starting on the following day) for three consecutive days, after which marked CBB females were no longer recaptured, as confirmed in previous trials (data not shown). The sticky cardboard liner was replaced and the alcohol reservoir was refilled after each evaluation. Marked CBBs (Figure 1C) stuck on the cardboard liners were counted with a stereoscope under ultraviolet light, rendering them fluorescent (Turchin and Odendaal, 1996; Meurisse and Pawson, 2017). The count of marked CBB females from a three-day collection was considered a replicate. The experiment was repeated five times during the postharvest period of coffee (i.e., lack of berries), on May 21 and 25 and June 4, 8, and 16 2019. This period was chosen to avoid any potential competitive effect of berries (i.e., visual and olfactive) (Vega et al., 2015) with the central alcohol trap on the attraction of released beetles.

The tests were conducted under clear skies with maximum air temperature of 14-26 °C and daily wind speed averaging 0.1 to 3.0 m s<sup>-1</sup> recorded from a weather station (Instituto Nacional de Meteorología - Santa Teresa) located approximately 18 km from the experiment site.

## Release-recapture data analysis

Recapture raw data of marked CBBs from the five replicates were analyzed by the linear and non-linear regression, using least squares as the fitting method. The



**Figure 1** – Illustration of methodology for single-trap, multiple-release-distance experiments: A) red four-unit funnel trap used as central semiochemical trap; B) multiple-release experiment design with color circles showing distances of releases at four cardinal directions of a central trap; C) coffee berry borers marked with fluorescent dust.

linear regression was specifically used to determine the relationship between the proportion of recaptures and release distances. Moreover, following the terminology and methodology of Miller et al. (2015), we constructed three scatter plots, from which non-linear fit curves were generated, including: 1) untransformed proportion of recaptured CBBs ( $spT_{fer}$ ) versus release distance from the central trap. In this case, when the fitted curve pattern was smoothly concave, approaching to zero catch asymptotically, the plot indicated that the release distances were adequately selected and that the target insect moved randomly in the field (Miller et al., 2015); 2) inverse proportion of recaptured CBB females ( $1 \text{ mean } spT_{fer}^{-1}$ ) versus release distance from the central trap, whose slope was used to determine the trap plume reach from plotted standard curves (i.e., Figure 4.12 in Miller et al., 2015); and 3) annulus area of release  $\times spT_{fer}$  versus release distance from the central trap. The resulting fit line was projected from the origin toward the intersection point on the x-axis to determine the 95 % maximum dispersal flight distance of the CBB population. As no marked CBBs were recaptured at 20 and 24 m (see the Results section), these distances were excluded from the analyses (Miller et al., 2015). The best-fit models considered satisfied the assumptions of normality of residuals (Shapiro-Wilk test), homoscedasticity of variance of residuals (homoscedasticity plot or test for appropriate weighting of the sum of squares), and with the highest  $R^2$ . All analyses and tests were made in GraphPad Prism version 10.0.2 for Windows (GraphPad Software).

As the plume reach could not be accurately estimated using standards curves (Miller et al., 2015;

see the Results section), we alternatively calculated the effective attraction radius (EAR), which measures the strength of attraction of a semiochemical trap, following the equation by Byers (1999):  $N_A = N_B \times (EAR/R \times \varpi)$ , where  $N_A$  is the predicted number of recaptured marked beetles,  $N_B$  is the number of released marked beetles within the trap radius,  $R$  is the trap radius (i.e., farthest release distance that resulted in capture) and EAR is the effective attraction radius (m) of the trap. In a Microsoft Excel spreadsheet, we simulated the effect of EAR on  $N_A$ . The EAR value best fitted to our data was determined by incrementing the EAR in steps of 0.01 from 0 until the value that resulted in the closest value of  $N_A$  to our observed recaptured data (Byers, 1999). The area of each trapping annulus was calculated by subtracting the inner radius squared from the outer radius squared and then multiplying by  $\varpi$  (Adams et al., 2017b). The trapping radius was calculated by summing the maximum dispersal flight distance and plume reach (EAR). The entire trapping area was calculated using the circle area equation, that is,  $\varpi \times \text{trapping radius squared}$ . The weighted average for the capture probability ( $T_{fer}$ ) of CBBs was calculated by dividing the mean annulus area  $\times spT_{fer}$  by the mean annulus area.

### Estimating the absolute CBB population density from trapping data

The absolute population density of CBBs ( $n^\circ$  colonizing females  $ha^{-1}$ ) was estimated from the total number of conspecific beetles caught per monitoring trap per ha within a trapping period of 3 d. For that, the number of trapped CBBs was initially divided by the trap area

(ha) and then by  $T_{fer}$  (Miller et al., 2015). Because CBB males are significantly outnumbered and do not leave the berries (Vega et al., 2015), they were not used for population density or crop damage estimation (below).

**Estimating coffee losses caused by CBB**

We estimated the damage to Arabica coffee production caused by the CBB in the region of Santa Teresa, where the experiments were carried out. Damage was based on the estimated CBB population density (see the Results section), as well as the average data on the intrinsic rate of natural increase ( $r = 0.045$ ) and generation time ( $T = 65.3$ ) at approx. 21.2 °C (Ruiz-Cárdenas and Baker, 2010), which corresponds to the average temperature in Santa Teresa from Jan (early filling of coffee beans) to May (harvest). Consequently, nearly two generations (F2) of the pest may occur during this period (Ruiz-Cárdenas and Baker, 2010). The net reproductive rate ( $R_0$ ) - the average number of females produced in two successive generations - was calculated for each generation following the formula in Birch (1948):  $R_0 = \exp^{(r \times T)}$ . The average number of individuals sustained per coffee berry is approximately 100 (Jaramillo et al., 2009). As berries harbor two seeds, the number of coffee beans bored by the insect per ha was obtained by dividing  $R_0$  by 50. From the average weight of 100 traded coffee beans with 12 % water content (15 g), we estimated that roughly 6667 beans yield 1 kg. Therefore, to express losses in kilograms of coffee beans per ha, we divided the number of bored beans by 6667.

**Results**

**Release-recapture of marked CBBs**

Overall, circa 97.7 % of the 2800 marked CBBs in each of the five replicates flew; they were not found dead either on or under the releasing platforms. From the seven release distances, recaptures by the central semiochemical-baited trap occurred until the radius of 16 m. The overall recapture of the dispersing beetles was 2.6 % (Table 1). Of these recaptures, 98.6 % occurred within the first 24 h after release, 1.4 % between 24 and 48 h, and no beetle was recaptured after this period (Table 1). Moreover, roughly 96 % of recaptures at release distances 2 and 4 m and 100 % at the remaining distances occurred within the first 24 h after release (Table 1). Additionally, there was a significant decrease in the proportion of recaptured beetles with the increase of the release distance ( $F = 266.8, df = 23, p < 0.0001$ ), with the linear regression model explaining nearly 92 % of the total variance of the recaptures (Figure 2). The mean ( $\pm$  standard error  $n = 5$  replicates) proportion of recaptured beetles for the closest release distance at 2 m was  $0.072 \pm 0.006$  and was  $0.005 \pm 0.001$  for the farthest distance of 16 m. The overall catch probability for all trapping annuli areas ( $T_{fer}$ ) was 0.01.

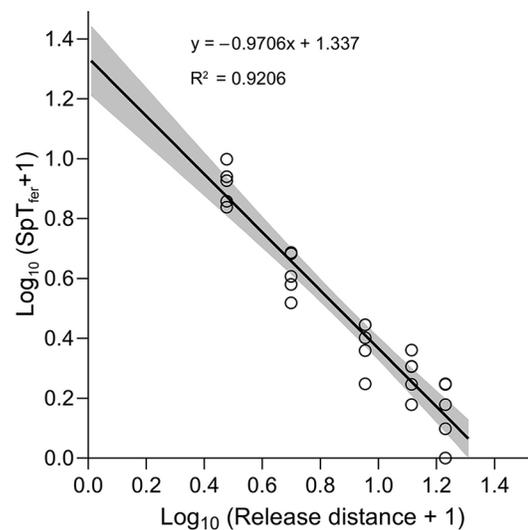
The non-linear fit curve for the untransformed proportion of recaptured CBBs by release distance was smooth concave, approaching the zero asymptote as the recaptures decreased with regular increases in the distance from the central alcohol trap (Figure 3A). The inverse proportion of recaptured CBBs by release distance (Figure 3B) was linear over the closest data points, with a steep slope (9.599) off the plot standard curve of Miller for the plume reach estimation. Alternatively, the estimated EAR of the alcohol trap was  $1.3 \pm 0.1$  m. Finally, the projected x-intercept for the second-order polynomial curve of the annulus area of release  $\times spT_{fer}$  by release distance showed that the 95 % maximum dispersal distance for the released CBBs was approximately 22.2 m (Figure 3C).

The sum of EAR and maximum dispersal flight distance equated to a sampling area for the present alcohol trap of about 1,734.9 m<sup>2</sup> or 0.17 ha.

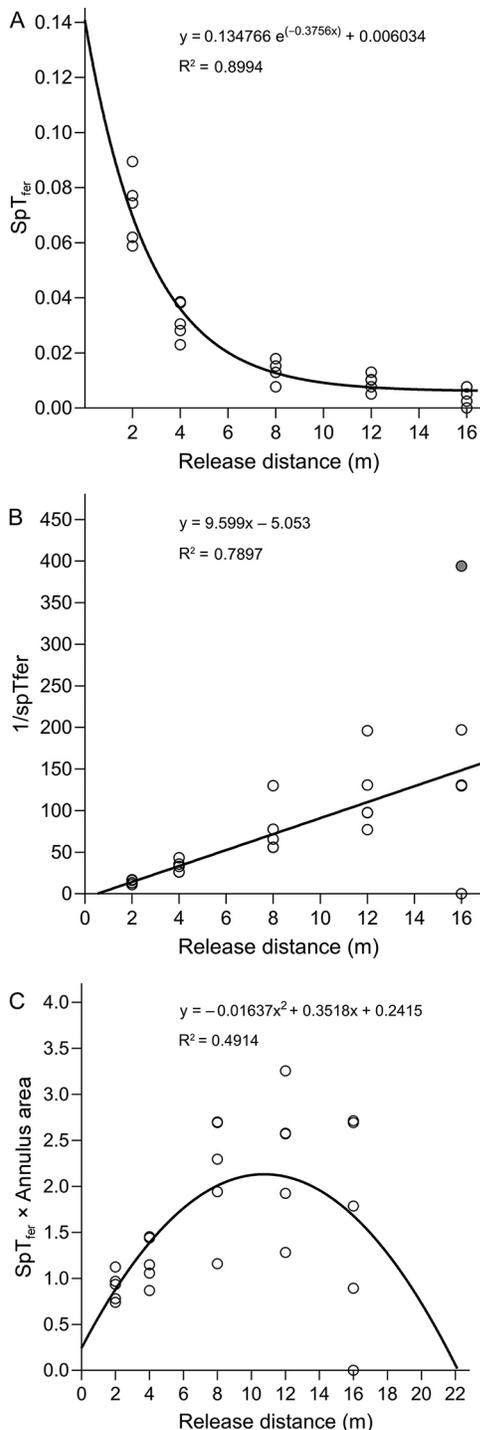
**Table 1** – Mean ( $\pm$  SE) number of marked coffee berry borers released at different distances from a central red 4-unit funnel trap baited with ethanol and methanol and percentage of recaptures per release distance and over time (N = 5 replicates).

Release distance (m)	N° of beetles released <sup>a</sup>	% of recapture	% recaptured over time after release (h) <sup>b</sup>		
			24	48	72
2	389.6 $\pm$ 0.7	7.2 $\pm$ 0.6	97.6 $\pm$ 1.7	2.4 $\pm$ 1.7	0
4	391.8 $\pm$ 0.9	3.2 $\pm$ 0.3	95.2 $\pm$ 2.0	4.8 $\pm$ 2.0	0
8	391.0 $\pm$ 1.0	1.4 $\pm$ 0.2	100	0	0
12	390.2 $\pm$ 1.1	0.9 $\pm$ 0.1	100	0	0
16	391.6 $\pm$ 1.1	0.5 $\pm$ 0.1	100	0	0

<sup>a</sup>Number of marked beetles (from 400 individuals) that flew from release platforms. <sup>b</sup>Calculated based on the total number of beetles recaptured for that distance.



**Figure 2** – Proportion of recapture ( $spT_{fer}$ ) of marked coffee berry borers at different release distances from a central ethanol-methanol-baited trap. Dependent and independent variables were logarithmically transformed to address the assumptions for linear regression. Solid line represents the best-fit model and the grey area shows 95 % confidence intervals.



**Figure 3** – Non-linear regression analyses of data from single-trap, multiple-release-distance experiments with marked coffee berry borers. A) Proportion of recapture ( $SpT_{fer}$ ) versus release distance (m) from a central, alcohol-baited trap; B) Inverse of recapture proportion by release distance; C)  $SpT_{fer} \times$  trap annulus area over release distance. Solid line in each panel represents the best-fit non-linear model. The grey dot in B shows the outlier deleted from the analysis using the GraphPad Prism outlier detector at  $Q = 1\%$  (Motulsky and Brown, 2006).

### Estimating the absolute CBB population density from trap data

From catches of 1, 10, and 100 colonizing CBB females per monitoring trap  $ha^{-1}$ , we estimated an absolute population density of 565, 5,653, and 56,530 individuals  $ha^{-1}$ , respectively.

### Estimating losses to coffee production caused by CBBs

From the initial population density of 56,530 colonizing CBB females  $ha^{-1}$  (see above) at the early filling stage of coffee beans, the estimated net reproductive rate ( $R_0$ ) value at coffee harvest (generation F2) was roughly 20.2 million borers  $ha^{-1}$ . Consequently, the estimated production loss of green coffee beans was around 60.3  $kg\ ha^{-1}$ .

## Discussion

The effective sampling area of the alcohol-baited multifunnel trap to monitor colonizing CBB females is approximately 0.17 ha. This parameter relied primarily on the maximum dispersal flight distance of CBBs within the trap radius rather than the reach of the trap plume. This pattern agrees with the low catch probability obtained in our study, which occurs when the net distance traveled by an insect is significantly longer than the reach of the odor plume (Miller, 2020). Here, we considered the EAR (Byers, 1999) as the plume reach of the alcohol trap. Our estimated EAR aligns with several other studies (i.e., < 2 m), regardless of the trap model and semiochemical type (Schlyter, 1992; Byers, 1999; Miller et al., 2015).

The short plume reach of ethanol and methanol might be related to their physicochemical properties. For example, plant-derived kairomones of low molecular weight, such as straight short-chain alcohols, produce odor-plume that diffuse and fade faster in the environment, hampering detection by the insect antennae far from the emission point (Gut et al., 2004). In addition, daily shifts in wind speed, temperature, and atmospheric turbulence levels can affect the odor plume structure (Elkinton et al., 1984; Cardé and Willis, 2008). For instance, as wind currents change speed and direction, vegetation obstacles, such as coffee trees, could break the semiochemical plume (Turchin and Odendaal, 1996). However, although we were unable to measure wind speed in the experimental locale, the nearest weather station recorded a relatively low wind speed during the daily flight peak of the CBB, that is, from late morning to mid-afternoon (Baker et al., 1992; Mathieu et al., 1997a). Besides, the wind speed was probably even lower inside the coffee plot (Johnson and Manoukis, 2021), which could have reached or approached optimal levels (approximately  $1\ m\ s^{-1}$ ) for diffusion and stability of the semiochemical plume (Miller et al., 2015).

We obtained an overall low recapture rate of the marked CBBs with catches decreasing as a function of the released distance from the central trap, which is line with most mark-release-recapture studies (Turchin and Odendaal, 1996; Byers, 1999; Dodds and Ross, 2002; Miller et al., 2015; Adams et al., 2017a, b; Meurisse and Pawson, 2017; Kirkpatrick et al., 2018, 2019). In fact, the number of marked CBBs released in the experiments was equivalent or superior to those used for other scolytine beetles (Turchin and Odendaal, 1996; Dodds and Ross, 2002). Moreover, we observed very low mortality before or during the releases, and it is unlikely that most of the "disappeared" marked CBBs died during the next three days of experiments. These beetles may survive ten days without food (Mathieu et al., 1997a) and find refuge in dry coffee berries or other coffee tree tissues (Vega et al., 2015). Additionally, mortality of marked CBBs due to the fluorescent dust has shown to be like non-marked beetles, for instance, only about 5 % 3 d after dusting (Acevedo-Bedoya et al., 2009). Besides, our line fitted for the untransformed proportion of recapture by the release distance revealed that the CBB moves randomly in the field and that the release distances were appropriately chosen (Miller et al., 2015). Finally, the overall catch probability in cardinal-direction releases has been like that of 11 releases (Adams et al., 2017b) thus indicating the suitability of our experimental design.

Therefore, in addition to the weak attraction power of the alcohol trap, two other important factors might have contributed to the low recapture rates. First, the coffee tree rows might have affected the dispersal direction of marked CBBs, as the beetles may have landed on these obstacles soon after taking off and then returned to flight in random directions (Byers, 1999). For instance, most recaptures occurred within the first 24 h after release, and all recaptures from distances 8, 12, and 16 m occurred during this period. Therefore, most beetles that flew had possibly already been dispersed out of the trap attraction range after 24 h (Schlyter, 1992). Second, we used newly-emerged CBBs for experiments, and most of them might have needed, although yet to be confirmed, a prior adaptative flight period to become responsive to the alcohol trap. For example, flight-experienced xylophagous and saprophytic scolytine beetles exhibited a relatively immediate chemotactic response to semiochemical traps compared to freshly-emerged beetles (Duelli et al., 1997; Byers, 1999; Meurisse and Pawson, 2017). Due to the higher reserves of lipids and glycogen in individuals of the last group, they may have extended the flight period and consequently dispersed outward the semiochemical trap vicinity (Duelli et al., 1997; Byers, 1999). This adaptive flight may last from minutes to hours, where it appears to have occurred the transition from phototactic to chemotropic orientation behavior (Duelli et al., 1997; Byers, 1999; Meurisse and Pawson, 2017).

Although coffee trees may shorten the duration of continuous flights, the dispersive capacity of the CBB appears relatively modest. For example, this beetle has been recorded infesting coffee berries on trees at 40 m from the release point after 24 h, under the same wind speed, similar to the findings in the present study (Gil et al., 2015). This corroborates laboratory observations on CBBs flying continuously for over 22 min before the first landing and displaying successive flights for up to 3 h (Baker, 1984). Furthermore, CBBs use coffee trees as bridges to disperse through connected coffee plots (Avelino et al., 2012), thus allowing these beetles to reach tens to hundreds of meters (Baker, 1984; Gingerich et al., 1996; Mathieu et al., 1999; Gil et al., 2015). Besides, the fact that a small percentage of CBBs have been trapped at heights between 2.5 and 3.5 m (Ruiz-Diaz and Rodrigues, 2021) also indicates that these beetles can fly upwards above coffee trees from where they could likely disperse to farther distances helped by stronger wind currents. However, the dispersive capacity of CBB still needs estimations, which could be addressed by releasing marked beetles in the center of concentric rings of semiochemical traps, where a variable number of traps per ring and distances from the release point are adopted (Turchin and Odendaal, 1996; Byers, 1999; Poland et al., 2000; Meurisse and Pawson, 2017). In addition, the first ring should have the lowest trap number and be appropriately spaced from the release point, avoiding thus trap interference by plume overlapping that could underestimate the recaptures and bias catches toward the closest rings (Turchin and Odendaal, 1996; Byers, 1999).

Nevertheless, our estimated sampling area suggests the use of approximately six traps  $\text{ha}^{-1}$  for CBB monitoring, which is lower than the average of nearly 19 traps  $\text{ha}^{-1}$  (range 2 – 59 traps  $\text{ha}^{-1}$ ) that has been reported elsewhere (Mathieu et al., 1999; Fernandes et al., 2011; Pereira et al., 2012; Aristizábal et al., 2015, 2017; Johnson et al., 2018; Souza et al., 2020; Johnson and Manoukis, 2020, 2021). This is economically desirable, as it reduces costs with traps, attractants, and labor time for sampling this coffee pest.

Another critical approach in the present study was the estimation of CBB population density using trapping data. We showed that capturing an average of one CBB monitoring per trap  $\text{ha}^{-1}$  equates to a most-probable population density of 565 conspecific beetles  $\text{ha}^{-1}$ . This result can be ascribed to the fact that most released marked CBBs were not recaptured, as reported above. Thus, trapping 100 CBBs  $\text{ha}^{-1}$  at the early filling stage of Arabica coffee beans, that is, 120 days after flowering (Ruiz-Cárdenas and Baker, 2010; Vega et al., 2015), corresponds to approximately 56,530 borers  $\text{ha}^{-1}$ . Consequently, the projected losses at harvest reaches roughly 4.5 %, considering the average Brazilian Arabica green coffee beans yield of around 1,330  $\text{kg ha}^{-1}$  (CONAB, 2022).

In Brazil, the economic threshold level for CBB is 4.3 % of infested coffee berries  $\text{ha}^{-1}$ , corresponding to 400 borers trap $^{-1}$   $\text{ha}^{-1}$  at the flowering period (Fernandes

et al., 2011). Our estimates, in contrast, indicate that this threshold may be reached with nearly 95 trapped CBBs ha<sup>-1</sup>, which equates to losses below 57 kg of green coffee beans ha<sup>-1</sup>. However, our estimations for damages to crop production still need conciliation with the actual progression of fruit infestation in the field (Baker and Barrera, 1993); therefore, the estimations should be cautiously adopted.

The present findings shed light on improvements to the use of alcohol traps and on the interpretation of catch data to enhance the integrated management of CBB. However, our study still needs refinement. Therefore, our experiments should be conducted in different coffee-growing systems and locations to estimate the corresponding trap sampling area. In addition, future studies on the performance of new attractants and trap designs for CBB monitoring are encouraged to adopt the methodology used in the present study.

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## Authors' Contributions

**Conceptualization:** Madalon FZ, Silva WD, Rainho HL, Bento JMS. **Methodology:** Madalon FZ, Silva WD. **Investigation:** Madalon FZ. **Formal analysis:** Silva WD. **Data curation:** Silva WD. **Funding acquisition:** Bento JMS. **Resources:** Bento JMS. **Project administration:** Bento JMS. **Supervision:** Bento JMS. **Writing-original draft:** Madalon FZ, Rainho HL. **Writing-review & editing:** Silva WD, Bento JMS.

## References

- Acevedo-Bedoya FE, Gil-Palacio ZN, Bustillo-Pardey AE, Montoya-Restrepo EC, Benavides-Machado P. 2009. Evaluation of physical and molecular markers as tools for the study of the dispersion of *Hypothenemus hampei*. *Cenicafé* 60: 72-85 (in Spanish, with abstract in English).
- Adams CG, McGhee PS, Schenker JH, Gut LJ, Miller JR. 2017a. Line-trapping of codling moth (Lepidoptera: Tortricidae): a novel approach to improving the precision of capture numbers in traps monitoring pest density. *Journal of Economic Entomology* 110: 1508-1511. <https://doi.org/10.1093/jee/tox147>
- Adams CG, Schenker JH, McGhee PS, Gut LJ, Brunner JF, Miller JR. 2017b. Maximizing information yield from pheromone-baited monitoring traps: estimating plume reach, trapping radius, and absolute density of *Cydia pomonella* (Lepidoptera: Tortricidae) in Michigan apple. *Journal of Economic Entomology* 110: 305-318. <https://doi.org/10.1093/jee/tow258>
- Aristizábal LF, Jiménez M, Bustillo AE, Trujillo HI, Arthurs SP. 2015. Monitoring coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae), populations with alcohol-baited funnel traps in coffee farms in Colombia. *Florida Entomologist* 98: 381-383. <https://doi.org/10.1653/024.098.0165>
- Aristizábal LF, Shriner S, Hollingsworth R, Arthurs S. 2017. Flight activity and field infestation relationships for coffee berry borer in commercial coffee plantations in Kona and Kau districts, Hawaii. *Journal of Economic Entomology* 110: 2421-2427. <https://doi.org/10.1093/jee/tox215>
- Avelino J, Romero-Gurdián A, Cruz-Cuellar HF, Declerck FAJ. 2012. Landscape context and scale differentially impact coffee leaf rust, coffee berry borer, and coffee root-knot nematodes. *Ecological Applications* 22: 584-596. <https://doi.org/10.1890/11-0869.1>
- Baker PS. 1984. Some aspects of the behavior of the coffee berry borer in relation to its control in Southern Mexico (Coleoptera, Scolytidae). *Folia Entomológica Mexicana* 61: 9-24.
- Baker PS, Ley C, Balbuena R, Barrera JF. 1992. Factors affecting the emergence of *Hypothenemus hampei* (Coleoptera: Scolytidae) from coffee berries. *Bulletin of Entomological Research* 82: 145-150. <https://doi.org/10.1017/S000748530005166X>
- Baker PS, Barrera JF. 1993. A field study of a population of coffee berry borer, *Hypothenemus hampei* (Coleoptera; Scolytidae) in Chiapas, Mexico. *Tropical Agriculture (Trinidad and Tobago)* 29: 656-662.
- Birch LC. 1948. The intrinsic rate of natural increase of an insect population. *The Journal of Animal Ecology* 17: 15-26. <https://doi.org/10.2307/1605>
- Byers JA. 1999. Effects of attraction radius and flight paths on catch of scolytid beetles dispersing outward through rings of pheromone traps. *Journal of Chemical Ecology* 25: 985-1005. <https://doi.org/10.1023/A:1020869422943>
- Cardé RT, Willis MA. 2008. Navigational strategies used by insects to find distant, wind-borne sources of odor. *Journal of Chemical Ecology* 34: 854-866. <https://doi.org/10.1007/s10886-008-9484-5>
- Companhia Nacional de Abastecimento [CONAB]. 2022. Monitoring of the Brazilian Coffee Crop: Fourth Survey = Acompanhamento da Safra Brasileira de Café: Quarto Levantamento. CONAB, Brasília, DF, Brazil. Available at: <https://www.conab.gov.br/info-agro/safras/cafe> [Accessed Jan 19, 2023] (in Portuguese).
- Dodds KJ, Ross DW. 2002. Sampling range and range of attraction of *Dendroctonus pseudotsugae* pheromone-baited traps. *The Canadian Entomologist* 134: 343-355. <https://doi.org/10.4039/Ent134343-3>
- Doležal P, Okrouhlík J, Davidková M. 2016. Fine fluorescent powder marking study of dispersal in the spruce bark beetle, *Ips typographus* (Coleoptera: Scolytidae). *European Journal of Entomology* 113: 111-114. <https://doi.org/10.14411/eje.2016.001>

- Duelli P, Zahradnik P, Knizek M, Kalinova B. 1997. Migration in spruce bark beetles (*Ips typographus* L.) and the efficiency of pheromone traps. *Journal of Applied Entomology* 121: 297-303. <https://doi.org/10.1111/j.1439-0418.1997.tb01409.x>
- Dufour BP, Frérot B. 2008. Optimization of coffee berry borer, *Hypothenemus hampei* Ferrari (Col., Scolytidae), mass trapping with an attractant mixture. *Journal of Applied Entomology* 132: 591-600. <https://doi.org/10.1111/j.1439-0418.2008.01291.x>
- Elkinton JS, Cardé RT, Mason CJ. 1984. Evaluation of time-average dispersion models for estimating pheromone concentration in a deciduous forest. *Journal of Chemical Ecology* 10: 1081-1108. <https://doi.org/10.1007/BF00987515>
- Fernandes FL, Picanço MC, Campos SO, Bastos CS, Chediak M, Guedes RNC, et al. 2011. Economic injury level for the coffee berry borer (Coleoptera: Curculionidae: Scolytinae) using attractive traps in Brazilian coffee fields. *Journal of Economic Entomology* 104: 1909-1917. <https://doi.org/10.1603/EC11032>
- Gil ZN, Benavides P, Souza O, Acevedo FE, Lima E. 2015. Molecular markers as a method to evaluate the movement of *Hypothenemus hampei* (Ferrari). *Journal of Insect Science* 15: 72. <https://doi.org/10.1093/jisesa/iev058>
- Gingerich DP, Borsa P, Suckling DM, Brun L. 1996. Inbreeding in the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae) estimated from endosulfan resistance phenotype frequencies. *Bulletin of Entomological Research* 86: 667-674. <https://doi.org/10.1017/S0007485300039183>
- Gut LJ, Stelinski LL, Thomson DR, Miller JR. 2004. Behaviour-modifying chemicals: prospects and constraints in IPM. p. 73-121. In: Koul O, Dhaliwal G, Cuperus G. eds. *Integrated pest management: potential, constraints and challenges*. CABI, Cambridge, MA, USA.
- Jaramillo J, Chabi-Olaye A, Poehling H, Kamonjo C, Borgemeister C. 2009. Development of an improved laboratory production technique for the coffee berry borer *Hypothenemus hampei*, using fresh coffee berries. *Entomologia Experimentalis et Applicata* 130: 275-281. <https://doi.org/10.1111/J.1570-7458.2008.00820.X>
- Johnson MA, Hollingsworth R, Fortna S, Aristizábal LF, Manoukis NC. 2018. The Hawaii protocol for scientific monitoring of coffee berry borer: a model for coffee agroecosystems worldwide. *Journal of Visualized Experiments* 133: e57204. <https://doi.org/10.3791/57204>
- Johnson MA, Manoukis NC. 2020. Abundance of coffee berry borer in feral, abandoned and managed coffee on Hawaii Island. *Journal of Applied Entomology* 144: 920-928. <https://doi.org/10.1111/jen.12804>
- Johnson MA, Manoukis NC. 2021. Influence of seasonal and climatic variables on coffee berry borer (*Hypothenemus hampei* Ferrari) flight activity in Hawaii. *PLoS One* 16: e0257861. <https://doi.org/10.1371/JOURNAL.PONE.0257861>
- Kirkpatrick DM, Gut LJ, Miller JR. 2018. Estimating monitoring trap plume reach and trapping area for *Drosophila suzukii* (Diptera: Drosophilidae) in Michigan tart. *Journal of Economic Entomology* 111: 1285-1289. <https://doi.org/10.1093/jeet/toy062>
- Kirkpatrick DM, Acebes-Doria AL, Rice KB, Short BD, Adams CG, Gut LJ, et al. 2019. Estimating monitoring trap plume reach and trapping area for nymphal and adult *Halymorpha halys* (Hemiptera: Pentatomidae) in crop and non-crop habitats. *Environmental Entomology* 48: 1104-1112. <https://doi.org/10.1093/ee/nvz093>
- Lindgren BS. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). *The Canadian Entomologist* 115: 299-302. <https://doi.org/10.4039/Ent115299-3>
- Mathieu F, Brun LO, Frérot B. 1997a. Factors related to native host abandonment by the coffee berry borer *Hypothenemus hampei* (Ferr.) (Col., Scolytidae). *Journal of Applied Entomology* 121: 175-180. <https://doi.org/10.1111/j.1439-0418.1997.tb01389.x>
- Mathieu F, Brun LO, Marchillaud C, Frérot B. 1997b. Trapping of the coffee berry borer *Hypothenemus hampei* Ferr. (Col., Scolytidae) within a mesh-enclosed environment: interaction of olfactory and visual stimuli. *Journal of Applied Entomology* 121: 181-186. <https://doi.org/10.1111/J.1439-0418.1997.TB01390.X>
- Mathieu F, Brun LO, Frérot B, Suckling DM, Frampton C. 1999. Progression in field infestation is linked with trapping of coffee berry borer, *Hypothenemus hampei* (Col., Scolytidae). *Journal of Applied Entomology* 123: 535-540. <https://doi.org/10.1046/j.1439-0418.1999.00400.x>
- Messing RH. 2012. The coffee berry borer (*Hypothenemus hampei*) invades Hawaii: preliminary investigations on trap response and alternate hosts. *Insects* 3: 640-652. <https://doi.org/10.3390/insects3030640>
- Meurisse N, Pawson S. 2017. Quantifying dispersal of a non-aggressive saprophytic bark beetle. *PLoS One* 12: e0174111. <https://doi.org/10.1371/journal.pone.0174111>
- Miller JR, Adams CG, Weston PA, Schenker JH. 2015. Trapping of small organisms moving randomly: principles and applications to pest monitoring and management. Springer, New York, NY, USA. <https://doi.org/10.1007/978-3-319-12994-5>
- Miller JR. 2020. Sharpening the precision of pest management decisions: assessing variability inherent in catch number and absolute density estimates derived from pheromone-baited traps monitoring insects moving randomly. *Journal of Economic Entomology* 113: 2052-2060. <https://doi.org/10.1093/jeet/toaa152>
- Mota LHC, Silva WD, Sermarini RA, Demétrio CGB, Bento JMS, Delalibera Junior I. 2017. Autoinoculation trap for management of *Hypothenemus hampei* (Ferrari) with *Beauveria bassiana* (Bals.) in coffee crops. *Biological Control* 111: 32-39. <https://doi.org/10.1016/j.biocontrol.2017.05.007>
- Motulsky HJ, Brown RE. 2006. Detecting outliers when fitting data with nonlinear regression - a new method based on robust nonlinear regression and the false discovery rate. *BMC Bioinformatics* 7: 123. <https://doi.org/10.1186/1471-2105-7-123>
- Oliveira CM, Santos MJ, Amabile RF, Frizzas MR, Bartholo GF. 2018. Coffee berry borer in conilon coffee in the Brazilian Cerrado: an ancient pest in a new environment. *Bulletin of Entomological Research* 108: 101-107. <https://doi.org/10.1017/S0007485317000530>
- Onufrieva KS, Onufriev AV, Hickman AD, Miller JR. 2020. Bounds on absolute gypsy moth (*Lymantria dispar dispar*) (Lepidoptera: Erebididae) population density as derived from counts in single milk carton traps. *Insects* 11: 1-17. <https://doi.org/10.3390/insects11100673>

- Onufrieva KS, Onufriev AV. 2021. How to count bugs: a method to estimate the most probable absolute population density and its statistical bounds from a single trap catch. *Insects* 12: 932. <https://doi.org/10.3390/insects12100932>
- Pereira AE, Vilela EF, Tinoco RS, Lima JOG, Fantine AK, Morais EGF, et al. 2012. Correlation between numbers captured and infestation levels of the coffee berry borer, *Hypothenemus hampei*: a preliminary basis for an action threshold using baited traps. *International Journal of Pest Management* 58: 183-190. <https://doi.org/10.1080/09670874.2012.676219>
- Poland TM, Haack RA, Petrice TR, Sadof CS, Onstad DW. 2000. Dispersal of *Tomicus piniperda* (Coleoptera: Scolytidae) from operational and simulated mill yards. *The Canadian Entomologist* 132: 853-866. <https://doi.org/10.4039/Ent132853-6>
- Ruiz-Cárdenas R, Baker P. 2010. Life table of *Hypothenemus hampei* (Ferrari) in relation to coffee berry phenology under Colombian field conditions. *Scientia Agricola* 67: 658-668. <https://doi.org/10.1590/S0103-90162010000600007>
- Ruiz-Diaz CP, Rodrigues JCV. 2021. Vertical trapping of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytinae), in Coffee. *Insects* 12: 607. <https://doi.org/10.3390/insects12070607>
- Schlyter F. 1992. Sampling range, attraction range, and effective attraction radius: Estimates of trap efficiency and communication distance in coleopteran pheromone and host attractant systems. *Journal of Applied Entomology* 114: 439-454. <https://doi.org/10.1111/j.1439-0418.1992.tb01150.x>
- Silva FC, Ventura MU, Morales L. 2006. Capture of *Hypothenemus hampei* Ferrari (Coleoptera, Scolytidae) in response to trap characteristics. *Scientia Agricola* 63: 567-571. <https://doi.org/10.1590/S0103-90162006000600010>
- Silva WD, Mascarin GM, Romagnoli EM, Bento JMS. 2012. Mating behavior of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae). *Journal of Insect Behavior* 25: 408-417. <https://doi.org/10.1007/s10905-011-9314-4>
- Souza RA, Pratisoli D, Araujo Junior LM, Pinheiro JA, Souza JFV, Madalon FZ, et al. 2020. *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae) answer to visual and olfactive stimuli in field. *Coffee Science* 15: e151656. <https://doi.org/10.25186/v15i.1656>
- Sullivan BT. 2005. Electrophysiological and behavioral responses of *Dendroctonus frontalis* (Coleoptera: Curculionidae) to volatiles isolated from conspecifics. *Journal of Economic Entomology* 98: 2067-2078. <https://doi.org/10.1093/jee/98.6.2067>
- Turchin P, Odendaal FJ. 1996. Measuring the effective sampling area of a pheromone trap for monitoring population density of southern pine beetle (Coleoptera: Scolytidae). *Environmental Entomology* 25: 582-588. <https://doi.org/10.1093/ee/25.3.582>
- Vega FE, Infante F, Johnson AJ. 2015. The genus *Hypothenemus*, with emphasis on *H. hampei*, the coffee berry borer. p. 427-494. In: Vega FE, Hofstetter RW. eds. *Bark beetles*. Elsevier, San Diego, CA, USA. <https://doi.org/10.1016/B978-0-12-417156-5.00011-3>
- Witzgall P, Kirsch P, Cork A. 2010. Sex pheromones and their impact on pest management. *Journal of Chemical Ecology* 36: 80-100. <https://doi.org/10.1007/s10886-009-9737-y>