ORIGINAL ARTICLE

Agricultural and Forest Entomology · Royal Entomological Society

Behavioural interactions between co-habiting females and their impact on productivity and offspring sex ratios in the coffee berry borer, *Hypothenemus hampei*

Ameka Myrie [1] | Peter Biedermann [2] | Jan Oettler [1] | Dwight Robinson [3] | Eva Schultner [1]

[1] Zoology/Evolutionary Biology, Universität Regensburg, Regensburg, Bavaria, Germany

[2] Forest Entomology and Forest Protection, Albert Ludwig University of Freiburg, Freiburg im Breisgau, Baden-Württemberg, Germany

[3] Department of Life Sciences, The University of the West Indies, Kingston, Jamaica

Correspondence
Ameka Myrie and Eva Schultner, Universitätsstr. 31, 93053 Regensburg, Germany.
Email: myriemeka@gmail.com and eva.schultner@gmail.com

Abstract

1. The coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) has invaded all coffee-producing regions of the world and causes substantial economic losses every year. A single female beetle typically infests one coffee berry, which her offspring consume over the course of development. Offspring then engage in sib-mating before daughters fly off to infest new berries.

2. We assessed infestation rates across different locations in Jamaica and found a strong positive correlation between double infestation and overall infestation rate, suggesting that females prefer to breed alone but will tolerate sharing a berry under resource constraints.

3. Using an artificial habitat to study productivity and behaviour in the laboratory, we found that single females produced significantly more offspring per capita than two females, suggesting that co-habitation is costly. Accordingly, co-habiting females spent a significant proportion of their time engaged in antagonistic interactive behaviours such as pushing. When daughter beetles were given the opportunity to stay with their mothers or to infest a new berry, they tended to leave.

4. In contrast to some related beetle species, which exhibit gregariousness and even cooperative behaviour, beneficial social interactions in the CBB appear to be limited to parental care.

KEYWORDS
beetle, competition, cooperation, fitness, pest insect

INTRODUCTION

Coffee (Rubiales: Rubiaceae: *Coffea arabica* (L., 1753) and *C. canephora* (Pierre ex A. Froehner, 1897)) is one of the most valuable traded crop plants in the world (Battista et al., 2016; Infante, 2018), generating approximately 6.346 million tons of coffee worldwide annually (Perez et al., 2023). The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) is the major insect pest of coffee, having spread to all coffee-producing regions of the world due to human traffic (Gauthier, 2010) and causing substantial economic losses every year (Oliveira et al., 2013; Vega et al., 2002). A fertilized female beetle infests a berry by boring an entry hole and then tunnelling through the coffee berry, where she makes galleries, and lays her eggs in the endosperm of the seed (Mariño et al., 2021). The mother and her offspring then consume the berry, with mothers showing parental care behaviours such as egg tending, similar to other Scolytinae bark beetles (Jordal et al., 2011).

Once female and male offspring hatch into adults, they mate within the berry, after which females leave to infest new, healthy berries (Infante et al., 2009), while the males remain in the coffee berry for the rest of their relatively short lives (Damon, 2000; Vega et al., 2014).

Typically, a berry is infested by a single female (Pereira et al., 2021). Commercial coffee species have a heterogeneous flowering pattern, and coffee farms can have 1–2 major flowering periods per year (Dufour et al., 2021; Myrie et al., 2023), meaning susceptible coffee berries are potentially available throughout the year (Myrie et al., 2023). Nevertheless, multiple females may infest the same berry (Kiran & Venkatesha, 2020). With multiple infestation comes an increased potential for competition between females, as a single berry provides limited space for oviposition and represents a fixed amount of food for offspring. Increasing beetle densities in artificial diet can decrease female fecundity, perhaps mediated by pheromones (Vega et al., 2011). Whether behavioural interactions between co-habiting females also play a role remains unknown because of the cryptic lifestyle of this calamitous pest.

We estimated multiple infestation levels of *H. hampei* in five localities across Jamaica to assess how common multiple infestation is under natural conditions. Beetles were then transferred to the lab, and using artificial diet sandwiches (Vega et al., 2017), we followed the life cycle of female beetles from infestation to the departure of daughters. To assess if multiple infestation influences fitness and to investigate whether co-habiting females engage in competition, we compared productivity and offspring sex ratios of single females and pairs of females. Productivity was furthermore compared between pairs of beetles from the same location and beetles from different locations, to test whether the origin of beetles, as a proxy for relatedness between females, influences fitness. Interactions between co-habiting females were observed, and female behaviour was quantified to identify potential cooperative or competition-associated behaviours. Finally, we observed mother–daughter interactions and assessed the propensity of daughters to leave their natal habitat.

MATERIALS AND METHODS

Frequency of double infestations

The number of berries with one and two CBB entry points was assessed on randomly chosen shrubs at two locations sampled for laboratory rearing, Hopewell (18.03674°N, 76.67991°W, $n = 10$ shrubs) and Mountain Spring (18.10905° N, 76.80392° W, $n = 10$ shrubs), along with three additional locations from the Blue Mountain region: Saint Clouds (18.03036°N, 76.67384°W, $n = 10$ shrubs), Rosehill (18.0806°N, 76.73849°W, $n = 10$ shrubs) and Clifton Ridge (18.07169°N, 76.69342°W, $n = 7$ shrubs). Three branches were assessed per shrub based on the researcher's height (one branch from the top at 1.37 m, one from the middle at 0.91 m and one from the bottom at 0.46 m); this was done to mitigate bias as the lower sections of the shrub have been shown to have higher percentage of infestations (Aristizábal et al., 1998). For each shrub, the number

of berries with one and two CBB entry points, as well as the total number of sampled berries, was noted and used to calculate the proportion of total infested berries among all sampled berries and the proportion of double infestations among all infested berries. The proportion of double-infested berries was compared between locations using a generalized linear model with binomial error structure, with the number of infested and non-infested berries per shrub as response variable and location as explanatory variable (function glm in R version 4.2.1). Model residuals were checked using residual tests implemented in the DHarma 0.4.6 (function testResiduals) in R version 4.2.1. A Pearson's correlation test was conducted to examine the relationship between the number of double-infested berries and the number of total infested berries per shrub (function cor.test in R version 4.2.1).

Rearing CBBs in the laboratory

CBBs were collected in December 2019 and January 2020 with permission of the Jamaica Agricultural Commodities Regulatory Authority. The beetles used in these experiments derived from Hopewell in the Blue Mountains (above 914.4 m.a.s.l) and Mountain Spring in the Highlands (between 457.2 and 914.4 m.a.s.l). Infested coffee berries (mature ripe and mature green berries with CBB entry points) were randomly picked from 12 coffee shrubs in Hopewell and 10 coffee shrubs in Mountain Spring, and brought back to the laboratory at the Universität Regensburg, Germany. Infested berries were opened in the laboratory, and developing stages (eggs, larvae, pupae) and adult beetles were transferred to individual glass vials containing approximately 10 mL artificial diet, following Vega et al. (2011), but with the addition of formaldehyde (formaldehyde solution min. 37% as a microbial inhibitor). The stock cultures were monitored weekly, and when stock cultures were too moist or too crowded, the beetles were transferred to a fresh culture using soft forceps. The vials of stock cultures were stored at 27°C/21°C and 100% humidity under LD 12:12 h photoperiod cycle in a dark climate chamber.

Examining the effects of double infestation on productivity and sex ratios

To examine the effects of double infestation on beetle fitness and behaviour, artificial diet sandwiches (Vega et al., 2017) were made using two-sided mounting tape, aluminium foil tape, two glass plates (each 5 cm × 5 cm × 1 mm) and artificial diet formed into discs. Each sandwich was sectioned into four chambers, and each chamber was provisioned with one 0.15 g disc of diet. Two dry beans in a coffee berry weigh approximately 0.3 g; thus, 0.15 g diet discs were used to represent one bean in order to mimic natural resource availability.

For productivity and sex ratio assessments, beetles from the Hopewell location were used. Single female beetles ($n = 38$) or pairs of female beetles ($n = 42$) were placed into individual chambers. These newly fertilized females were collected from the stock vials

TABLE 1 Description of the behaviours observed between cohabiting females in artificial diet sandwiches over 12-h periods.

	Behaviour	Description
Interactive	Avoidance	Females are in contact; one female or both move away, avoiding the interaction after which females may resume touching, pushing or pulling or partake in another behaviour such as digging or escaping.
	Pushing/ pulling	Females are in contact; however, there is force or aggression in the action. Typically, head-to-head or head-to-abdomen pushing and pulling (pulling of the legs of one female by another can also be observed).
	Touching	Females are in contact; however, there is no sign of force or aggression in the action.
Non-Interactive	Digging–escaping	One female is digging or can be seen in the diet, whereas the other female can be seen biting through or standing by the two-sided tape*.
	Both digging	Both females are digging through the diet but there is no contact.
	Both escaping	When both females are standing or digging through the two-sided tape*.

*Two-sided tape is used to close the breeding containers.

based on their location in the vial: newly fertilized females actively relocate to the upper sections of the vial, while mothers remain buried in the diet. Single females were taken from one of two different stock vials. Pairs of females placed in the same chamber were always taken from the same stock vial, with a total of eight stock vials used.

The artificial diet sandwiches were stored in a climate chamber under the same conditions as the stock vials for 5 weeks, after which the chambers were observed using a microscope, and the number of offspring (eggs, larvae, pupae and young adults) produced was noted and used to assess productivity. The per capita data set was generated from the same data set; however, to get a more accurate comparison between each female from either treatment, the total number of offspring in chambers with pairs of females was divided by 2 and the chambers with only one female remained unchanged.

After assessment of productivity, a subset of sandwiches ($n = 23$ chambers containing single females, $n = 27$ chambers containing pairs) were returned to the climate chamber for an additional 2 weeks. After a total of 7 weeks, the artificial diet sandwiches containing the live beetles were frozen at $-80°C$ for at least 4 h, after which the glass plates of each sandwich were taken apart, and the adult female and male offspring in each chamber were counted under a dissecting microscope to assess offspring sex ratio. To test for differences in overall productivity and sex ratio between beetles kept alone

or in pairs, linear models, generalized linear models and analysis of variance (ANOVA) tests were done (function lm, glm, testResiduals and ANOVA) in R version 4.2.1. Model residuals were checked using residual tests implemented in the DHarma 0.4.6 package (function testResiduals) in R Version 4.2.1.

Examining the effects of double infestation on behaviour

To characterize beetle behaviour and to determine the best method to quantify behaviours, beetles in artificial diet sandwiches, stock vials and Petri dishes were placed under a microscope and observed daily for 15-min periods over the course of 3 weeks, before setting up experiments. From these observations, a total of seven behaviours were identified: avoidance, pushing/pulling, touching, digging–escaping, both digging and both escaping (Table 1).

To quantify beetle behaviour, two beetles from the same location (Hopewell–Hopewell: $n = 20$, Mountain Spring–Mountain Spring: $n = 19$) or two different locations (Hopewell–Mountain Spring: $n = 20$) were placed into a chamber. Each pair of females from the same location was taken from the same stock vial, with three stock vials used per location. Pairs of beetles from different locations were taken from a total of three stock vials per location. To be able to track female beetles, the elytra of the beetles were marked with enamel paint according to location before being added to the chambers of the artificial diet sandwiches; this did not appear to have adverse effects on the beetles' activity. Beetles from the same location were always marked with the same colour enamel paint. The colours and the order of treatment setup were randomized to avoid bias. The artificial sandwiches were then recorded using a Dinolite am4815zt edge microscope camera (Sotac Computer GmbH, Germany). For filming, the artificial diet sandwich was placed on a 6 cm × 7 cm × 4 cm white Styrofoam. A clamp was used to hold both the camera and the artificial diet sandwich on the Styrofoam in place. A fluorescent illuminator covered with pink foil was used as a source of minimum light and to prevent the production of heat, which could cause the diet to dry faster resulting in a decrease in beetle activity. The camera was able to capture the entire sandwich with a standard working distance and its range of magnification, providing good quality videos in low-light conditions. After each recording session, the sandwiches were returned to the climate chamber.

The recordings were loaded into the Avidemux software (version 2.7.6), where they were cropped into hourly video sessions and downscaled from 10 to 200 frames per second. Three 12-h recordings were produced for each chamber: (1) 12 h after setup (starting 30–60 min after artificial diet sandwich setup), (2) 1 week after setup and (3) 2 weeks after setup. The frequency and duration of each behaviour were quantified separately for each of the three time points using JWatcher [version 17.0.2 (8.86)]. To test whether behaviour frequency and duration were correlated, correlation tests were conducted using Pearson's correlation (cor.test in R version 4.2.1). There was a strong positive correlation between frequency and duration for

all behaviours across time points and populations; therefore, we focused on frequency for all further analyses (Figure S2). The frequency of each behaviour was compared between the three time points and between treatments. In addition, we grouped behaviours as interactive (avoidance, pushing/pulling, touching) and non-interactive (digging–escaping, both escaping, both digging) (Table 1). To test for differences between the frequencies of behaviours across the different time periods and treatments, Kruskal–Wallis rank sum tests were performed (function kruskal.test) in R version 4.2.1. This was done once for all interactive behaviours combined, as well as for each behaviour separately. Pairwise differences were identified using a Dunn's test (function dunn.test, method = "bonferroni") in R version 4.2.1.

After 5 weeks, total productivity in a subset of chambers used for behavioural observations was documented (intralocation: Hopewell–Hopewell: $n = 10$, Mountain Spring–Mountain Spring: $n = 18$, interlocation: Hopewell–Mountain Spring: $n = 14$). Differences in overall productivity between beetle pairs were analysed using a generalized linear model (function glm, in R version 4.2.1), and model fit was checked using residual tests implemented in the DHarma 0.4.6 package (function testResiduals in R version 4.2.1). To test for an association between social interactions and fitness, Spearman's rank correlation tests were run between each behaviour and total productivity (function cor.test in R version 4.2.1).

Testing for social interactions between mothers and daughters

Potential interactions between mothers and daughters were documented using artificial diet sandwiches sectioned into two equal chambers separated by a piece of two-sided tape (6 mm × 10.5 mm × 0.3 mm) and an aluminium bar (6 cm × 1 mm × 0.3 mm). Each chamber was provisioned with a 0.3 g artificial diet disc. A single female was placed in one chamber of the sandwich with one of the diet discs ("natal diet" disc). Females were taken from a total of five stock vials from the Hopewell location. In each chamber, offspring production was monitored weekly for 4 or 5 weeks, that is, until F1 females were fully developed ($n = 12$). Following full sclerotization and wing development of F1 females, their behaviour was documented as solitary (no interaction with mother or siblings) or social (interaction with mother or siblings) once every 15 min for 1 h per day for 6 days. Observations were made at the start of the hour, after 15 min, after 30 min, after 45 min and after 60 min, for 6 days after daughters were fully developed. The sample size decreased over the duration of the observations due to the mortality of mothers [days 1–4 ($n = 14$), day 5 ($n = 13$), day 6 ($n = 12$)]. The aluminium bar was then removed, and the number of fully developed females in the natal diet and in the new diet disc was recorded weekly for 2 weeks. A generalized linear model was used to test whether the number of females present in the natal diet changed over time (function glm in R version 4.2.1). Model residuals were checked using residual tests implemented in the DHarma 0.4.6 package (function testResiduals) in R version 4.2.1.

RESULTS

Double infestations increase with overall infestation rate in the field

A total of 4125 berries were sampled across five locations, of which 18.62% (768/4125) were infested (Table 2). Of the total infested berries, 8.07% (62/768) were double-infested. Double-infested berries refer to berries with two entry points, that is, either two entry points on one side of the coffee berry pinhole (more frequent) or two entry points on opposite sides of the coffee berry pinhole.

The proportions of double-infested berries varied between shrubs across locations (Figure 1a, GLM, factor: location, $F_{4,47} = 3.559$, $p < 0.001$). The number of double-infested berries per shrub increased with the number of total infested berries (Figure 1b, Pearson's correlation, $R^2 = 0.642$, $p < 0.001$).

Double infestation affects per capita productivity

Overall, two females produced more offspring than single females (Figure 2a, ANOVA, factor: treatment, $F_{1,80} = 4.305$, $p = 0.040$). However, per capita, single females produced significantly more offspring than two females [Figure 2b, ANOVA, factor: treatment, $F_{1,80} = 7.569$, $p = 0.007$; for results by developmental stage (eggs, larvae, pupae, young adults) see Figure S1].

Two females also tended to produce more males than single females (Figure 3a, ANOVA, factor: treatment, $F_{1,50} = 2.746$, $p = 0.104$), but per capita production of males was similar (Figure 3b, ANOVA, factor: treatment, $F_{1,50} = 2.970$, $p = 0.091$). There was no difference in the proportion of male offspring produced by single females and pairs of females (Figure 3c, GLM, factor: treatment, $F_{1,50} = 0.002$, $p = 0.965$).

Social interactions between founding females may explain decreased per capita productivity

When two females from the same or different location were allowed to infest artificial sandwiches together, they produced similar numbers of offspring (Figure 4, ANOVA, factor: treatment, $F_{2,42} = 2.06$, $p = 0.141$), demonstrating that origin has no effect on productivity. Behavioural observations revealed that co-habiting females engaged in social interactions, particularly touching and pushing, and that the frequency of social interactions varied over time (Figure 5, all interactive behaviours combined: Kruskal–Wallis rank sum test, $\chi^2 = 212.76$, df $= 2$, $p < 0.001$, Table S1). The frequency of interactive behaviours also differed between the three treatments (all interactive behaviours combined: Kruskal–Wallis rank sum test: $\chi^2 = 108.9$, df $= 2$, $p < 0.01$, Table S2), but there was no consistent effect of treatment, as both the highest and lowest frequencies of interactive behaviours (summed over all time points) occurred in the intralocation setups (194 occurrences in Hopewell setups, 672 occurrences in

TABLE 2 Overview of sampled coffee berries and CBB infestation levels across five locations in Jamaica.

| Location	Total number of sampled shrubs	Total number of sampled berries	Total infested berries (%)	Double-infested berries in Total infested berries (%)	Mean number of infested berries per shrub (±SD)	Mean number of double-infested berries per shrub (±SD)
Clifton Ridge	7	848	15.33 (130/848)	11.54 (15/130)	18.57 ± 10.98	2.14 ± 1.46
Hopewell	10	1134	29.98 (340/1134)	5.88 (20/340)	34 ± 21.67	2 ± 2.31
Mountain Spring	10	264	23.48 (62/264)	14.52 (9/62)	6.2 ± 3.58	0.9 ± 1.2
Rosehill	10	872	9.17 (80/872)	16.25 (13/80)	8 ± 8.72	1.3 ± 2.87
Saint Clouds	10	1007	15.49 (156/1007)	3.21 (5/156)	15.6 ± 10.47	0.5 ± 0.97
All locations combined	47	4125	18.62 (768/4125)	8.07 (62/768)	16.34 ± 15.89	1.32 ± 1.95

Abbreviation: CBB, coffee berry borer.
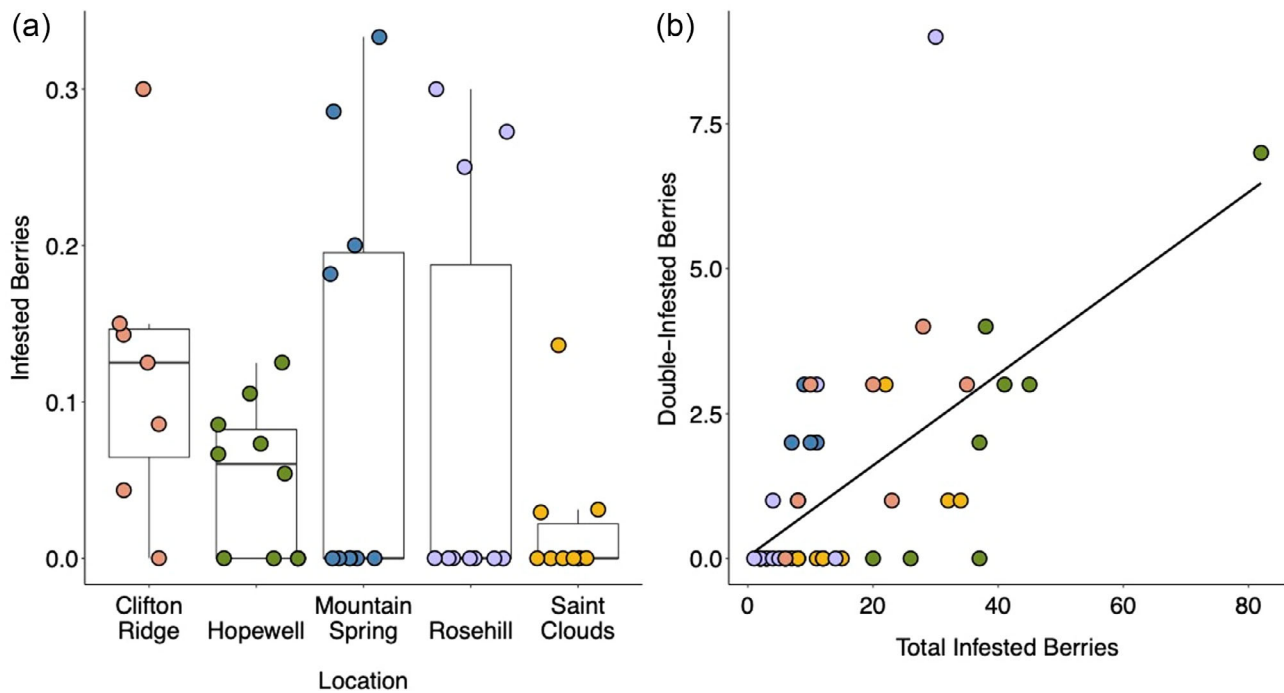
FIGURE 1 Double infestation of berries by the coffee berry borer *Hypothenemus hampei* occurs at the five locations sampled in Jamaica. (a) Proportion of double-infested berries per shrub. Boxplots show medians and upper and lower quartiles. (b) The number of double-infested berries increases with the overall number of infested berries per shrub.

Mountain Spring setups), whereas the overall frequency was intermediate in the interlocation treatment (436 occurrences). Similarly, the frequencies of each individual behaviour varied over time (Table S3) and between treatments (Table S4). During observations, bruises were occasionally observed on larvae; these were brown in colour and often seen within the upper region of the body.

There were no significant correlations between the frequency of behaviours and the total number of offspring produced in the three treatments, neither when each behaviour was analysed separately (Figure 6 and Table S5) nor when behaviours were grouped as interactive (Pearson's correlation, df = 124, $R^2 = 0.054$, $p = 0.551$) and non-interactive (Pearson's correlation, df = 124, $R^2 = 0.046$, $p = 0.605$). There was a consistent trend showing that

total productivity increased with the frequency of both females digging.

Daughters do not remain with mothers

From day 1 to day 6 after full development, fully developed daughters only engaged in social interactions in 5.43% of cases (22 out of 405 total 15-min observations). One week after hatching, 94% (64/68) of fully developed daughters remained in their natal diet. Two weeks after hatching, an additional 84 daughters were fully developed, increasing the total to 152 fully developed daughters. Of these, 65% (99/152) remained in their natal diet 2 weeks after hatching. The
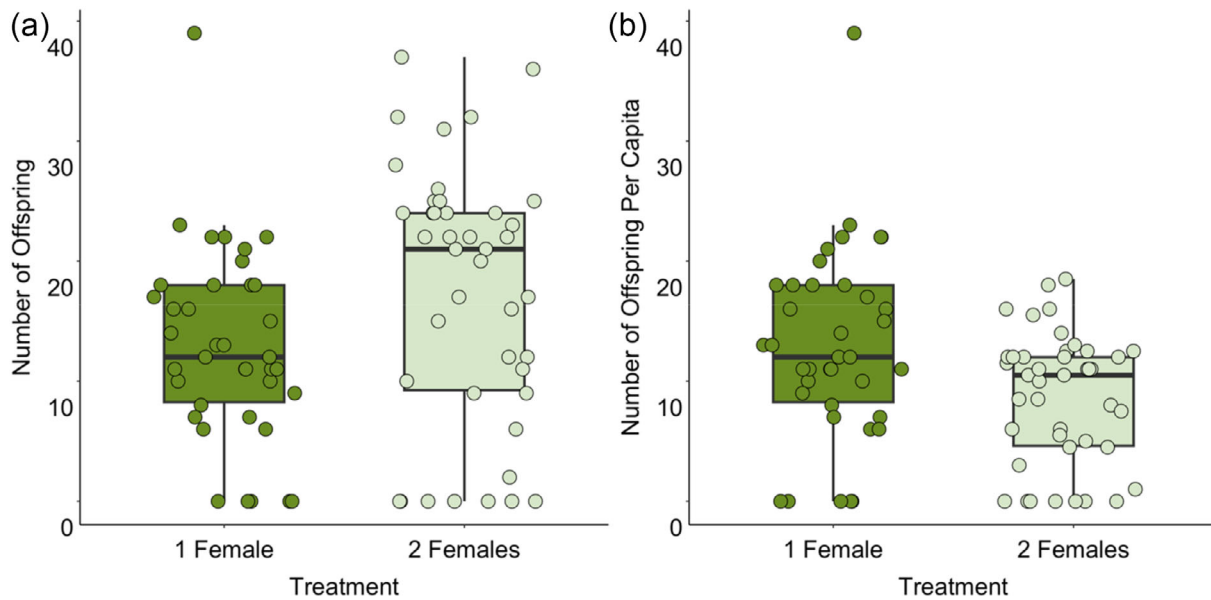
FIGURE 2 Productivity of single females and pairs of females of the coffee berry borer *Hypothenemus hampei*. (a) Number of offspring produced after 5 weeks (b) Number of offspring per capita produced after 5 weeks. Boxplots show medians and upper and lower quartiles.
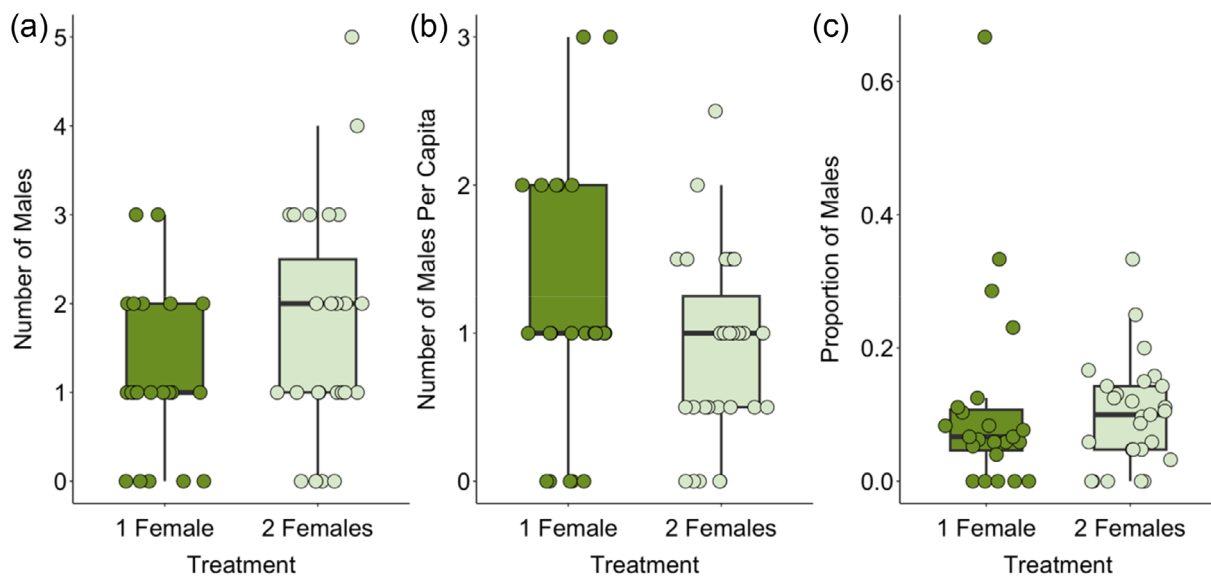

FIGURE 3 Male production by single females and pairs of females of the coffee berry borer *Hypothenemus hampei*. (a) Number of males produced after 7 weeks. (b) Number of males produced per capita after 7 weeks. (c) Proportion of adult males among all adult offspring produced after 7 weeks. Boxplots show medians and upper and lower quartiles.

proportion of fully developed daughters remaining in their natal diet decreased significantly over time (Figure 7. GLM, $F_{1,12} = 26.172$, $p < 0.001$).

DISCUSSION

Coffee is a major commodity on the island of Jamaica (Birthwright, 2016), but production has decreased significantly over the years due to factors including pests and diseases (Birthwright &

Barker, 2015; Statistical Institute of Jamaica, 2018). The CBB was first discovered in Jamaica in 1978 (Rhodes & Mansingh, 1986), and today it is present on all coffee farms across the island, causing losses in yield that are detrimental to approximately 80,000 Jamaicans who depend on coffee as their source of income (Guido et al., 2020). The invasion success of the CBB can be explained by a high rate of reproduction, with females producing over 100 eggs within a month of entering a new berry (Jaramillo et al., 2009), coupled with sib-mating and a protected life cycle within the berry in large monocultures. In the field, a single female beetle and her offspring can consume a berry

CONSEQUENCES TO CO-HABITATION IN CBBS

Agricultural and Forest
Entomology

Royal
Entomological
Society

7

within weeks of infestation, demonstrating that berries represent a fixed and limited amount of food. Although single infestations appear to be the norm in the field, double infestations occur with increasing

beetle densities (Johnson et al., 2020), potentially leading to competition over berries.

Allee effects are common in related bark and ambrosia beetles that jointly overcome difficulties such as host defences and/or manage growth of microbes (Gugliuzzo et al., 2023; Raffa, 2001). Beetles from the genus *Ambrosiodmus* (Coleoptera: Curculionidae), Hopkins, 1915 and *Ambrosiophilus* (Coleoptera: Curculionidae), Hulcr and Cognato 2009, for example, form communal colonies within fungus-colonized wood material (Kasson et al., 2016). In the absence of an allee effect, an increase in the number of individuals on a substrate would generally result in reduced fitness for each individual. In our experiments with artificial diet corresponding to one coffee bean, per capita productivity was lower for co-habiting beetles compared with single founding females. This confirms a previous study showing signs of competition even when resources are not limited (Vega et al., 2011). CBBs appear to mark berries with volatile pheromones, which repels other CBBs from entering (Njihia et al., 2014), perhaps as a means of preventing such competition. This is in contrast to other gregarious bark beetles that produce aggregation pheromones to jointly infest and overcome host trees (Raffa, 2001). Together with data from the field showing a positive correlation between overall infestation levels and double infestation, our results thus indicate that double infestation is a consequence of resource limitation which can incur fitness costs for individual females.

Behavioural observations revealed that decreases in productivity of co-habiting CBBs may result from increased time spent in social interactions. Beetles frequently engaged in antagonistic interactive
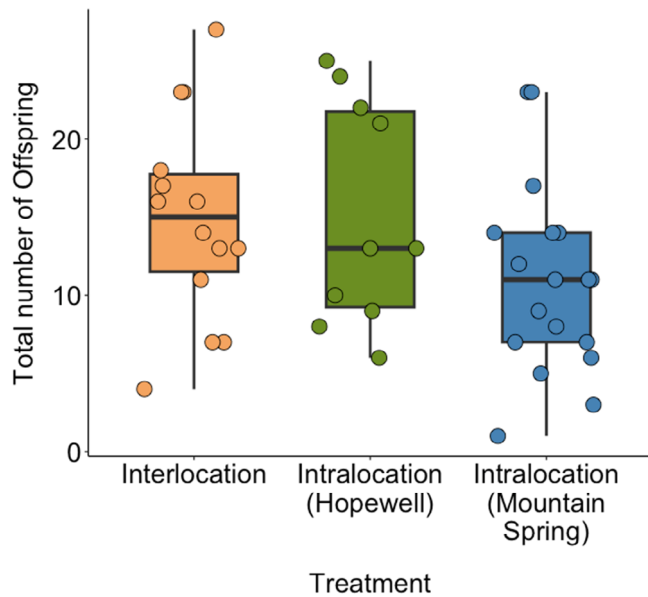

FIGURE 4 Differences in productivity based on female origin and cohabitation. Boxplots show medians and upper and lower quartiles.
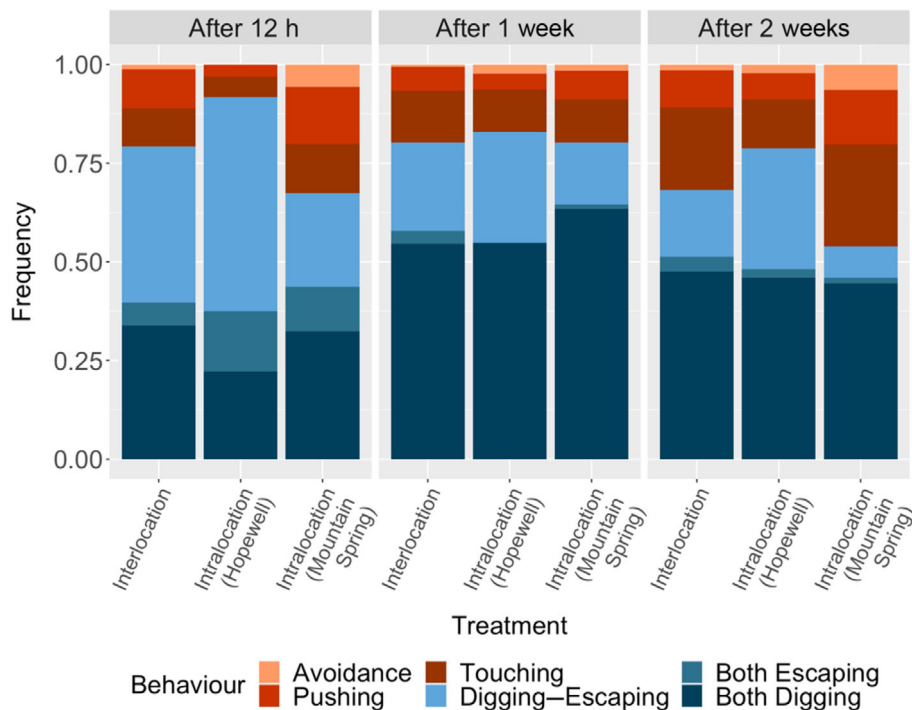

FIGURE 5 The frequencies of behaviours shown by co-habiting females vary over time and between treatments. Frequencies of behaviours are shown as proportions and behaviours are colour-coded as interactive (orange–red: avoidance, red: pushing/pulling, wine red: touching) and non-interactive (light blue: digging–escaping, dark blue: both escaping, deep blue: both digging). N for each time point (12 h, 1 week, 2 weeks) and treatment: interlocation (24, 20, 21); intralocation: Hopewell (22, 20, 19); intralocation: Mountain Spring (22, 20, 20).
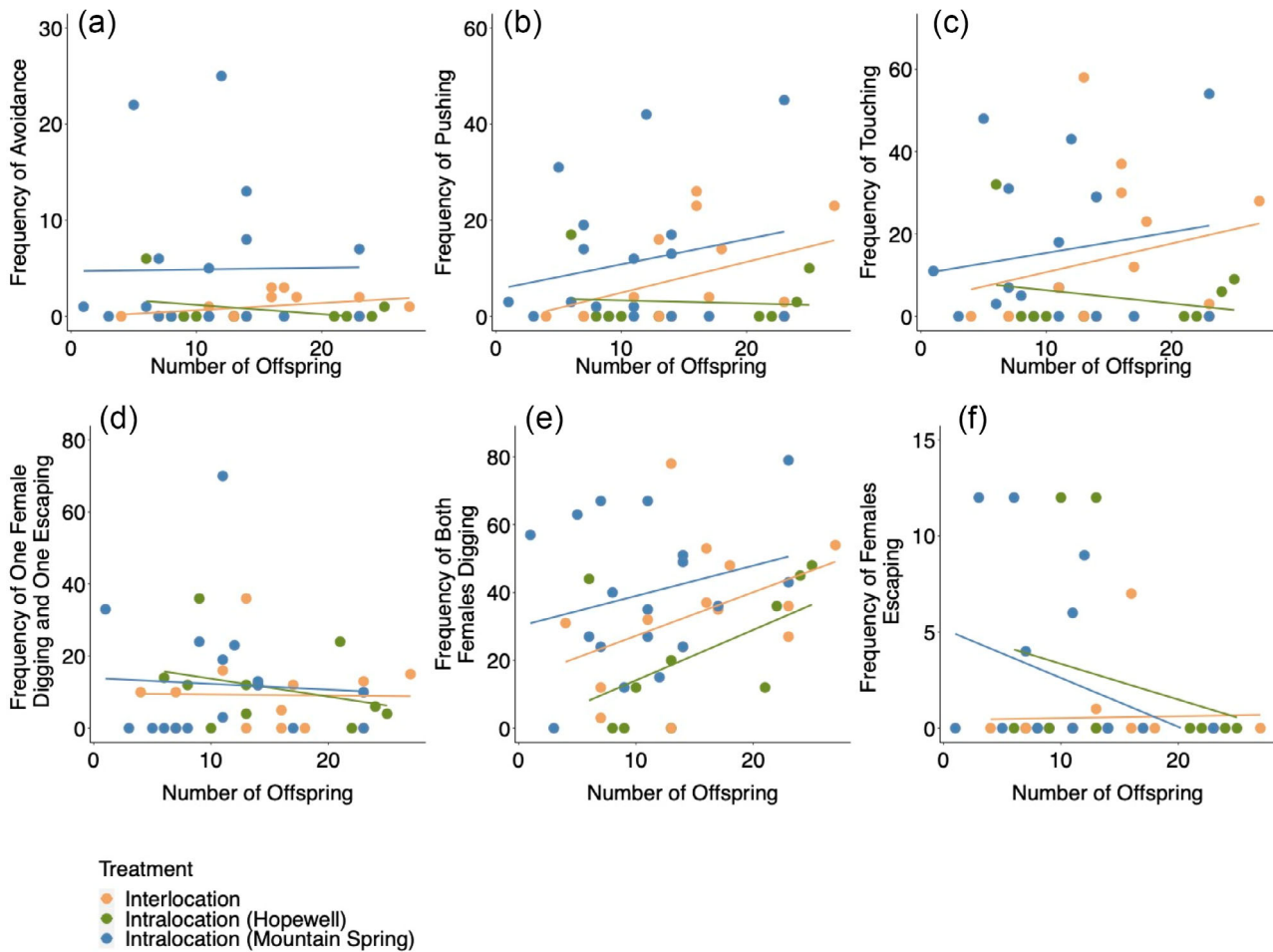
FIGURE 6 Relationship between the frequency of recorded behaviours and productivity of pairs of the beetles from the same or different locations. Behaviours are classified as interactive: avoidance (a), pushing/pulling (b), touching (c) and non-interactive: digging–escaping (d), both digging (e), both escaping (f).

behaviours such as pushing and pulling immediately after entering fresh diet, and the frequency of interactive behaviours increased over time. More time spent in social interactions means less time digging, so that beetles may produce fewer offspring simply due to lack of time. Although there was no statistically significant relationship between the frequency of social interactions and total offspring number, beetles from Mountain Spring, which showed the highest frequency of interactive behaviours, also tended to produce fewer offspring. Reduced offspring numbers may furthermore result from altercations due to the presence of more larvae, leading to injuries and potentially higher mortality during development. Competition between females also became apparent in gallery-blocking behaviour, which was commonly observed after oviposition, with the non-reproducing female trying to enter the gallery of the reproducing female. Similar behaviours are exhibited by female ambrosia beetles during brood care (Biedermann & Taborsky, 2011).

Competition between co-founding CBBs may be limited to effects on overall productivity, as double infestation did not affect offspring sex ratios, with both single beetles and beetle pairs producing a median proportion of ~10% males. With local mate competition, sex
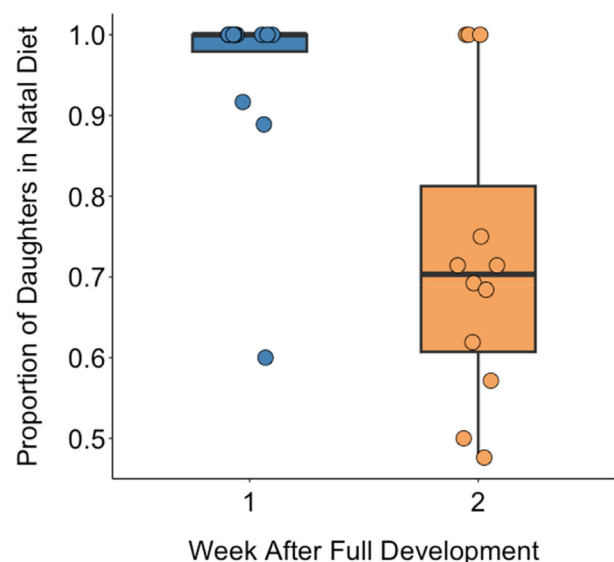

FIGURE 7 Proportion of daughters remaining in their natal diet after completing development. Boxplots show medians and upper and lower quartiles.

ratios are expected to be female biased, but theory suggests that more males are produced if there are outbreeding opportunities (Gottlieb et al., 2014). Competition between reproducing females may also lead to increased investment into sons, although high relatedness between females can mitigate such effects (Hamilton, 1967). For example, in the palm seed borer beetle, single females and pairs of related females produced female-biased sex ratios, whereas unrelated females increased production of males (Gottlieb et al., 2010). In our experiments, both single females and pairs of related females (i.e., from the same location) produced highly female-biased sex ratios, confirming results from previous studies (Vega, 2008). Whether unrelated females adjust offspring sex ratios in the CBB remains unclear, as the effect of female relatedness was only investigated in regards to overall productivity and behaviour. There were no consistent differences in productivity or frequency of interactive behaviours between related (i.e., intralocation) and unrelated (i.e., interlocation) female pairs, suggesting that females do not discriminate between phenotypes from different locations, perhaps because of a lack of informative cues about population origin.

Sociality can be observed in some Scolytinae beetles, most often associated with delayed dispersal by daughters and sometimes coupled with alloparental brood care (Biedermann, 2020; Biedermann et al., 2011; Khanday & Buhroo, 2020). Eusociality as defined by reproductive division of labour, cooperative brood care and overlapping generations has only evolved in ambrosia beetles, likely facilitated by female-biased sex ratios together with haplodiploidy and high within-group relatedness (Biedermann & Taborsky, 2011). These traits also occur in the CBB; however, there was limited evidence for cooperative within-family interactions in our experiments. Daughters exhibited slight delays in dispersal, but this was probably due to maturation feeding. Although observations over longer time periods may have yielded additional examples of social behaviour, the steady rate of dispersal by daughters appears to confirm the solitary lifestyle of this beetle.

Negative effects of high population density on reproductive output have been documented in many species, including in insect pests such as the Spruce Pine Beetle (Anderbrant et al., 1985). In some cases, this can lead to the collapse of invasive populations. For example, populations of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae), Mayr, 1868, have experienced collapse in invaded areas, possibly as a result of the combination of low genetic diversity, depletion of resources and pathogen pressure (Cooling et al., 2011). In contrast, population collapse does not appear to be an issue for the CBB, which benefits from monocultures and bad management practices. Nevertheless, the results of our study can help inform management practices. In particular, our results indicate that when uninfested berries are scarce, females are more willing to cohabit, in spite of negative consequences for individual fitness. This suggests that (1) with limited resources, there should be more CBBs actively flying around and (2) females are less choosy when infestation rates are high, which could mean that they may also be more easily attracted to traps. Indeed, CBBs were most active based on trap catches in periods of limited susceptible berries (Myrie et al., 2023). Regularly assessing the presence of multiple entry holes in berries in the field may thus be one way of efficiently timing the implementation of mechanical control strategies such as traps. This confirms that a better understanding of the basic biology of the beetle, along with knowledge of the ecological and environmental factors contributing to its success at different spatial scales (Vilchez-Mendoza et al., 2022), will contribute to improving the management of this important pest.

CONFLICT OF INTEREST STATEMENT

All authors declare that there is no conflict of interest related to the research presented in this study.

DATA AVAILABILITY STATEMENT

The raw data tables used to generate all the figures and statistics included in the manuscript are available upon request from the corresponding authors.

ORCID

Ameka Myrie https://orcid.org/0009-0009-2042-930X
Jan Oettler https://orcid.org/0000-0002-8539-6029
Dwight Robinson https://orcid.org/0000-0002-2286-127X
Eva Schultner https://orcid.org/0000-0002-5069-9732

REFERENCES

Anderbrant, O., Schlyter, F. & Birgersson, G. (1985) Intraspecific competition affecting parents and offspring in the bark beetle *Ips*

typographus. *Oikos*, 45(1), 89–98. Available from: https://doi.org/10.2307/3565226

Aristizábal, L., Bustillo, A., Orozco, J. & Chaves, C.B. (1998) Effect of parasitoid *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae) on populations of *Hypothenemus hampei* (Coleoptera: Scolytidae) during and after the harvest. *Revista Colombiana de Entomologia*, 24, 149–155.

Battista, F., Fino, D. & Mancini, G. (2016) Optimization of biogas production from coffee production waste. *Bioresource Technology*, 200, 884–890. Available from: https://doi.org/10.1016/j.biortech.2015.11.020

Biedermann, P.H.W. (2020) Cooperative breeding in the ambrosia beetle *Xyleborus affinis* and management of its fungal symbionts. *Frontiers in Ecology and Evolution*, 8, 518954. [Accessed July 7th 2023] Available from: https://www.frontiersin.org/articles/10.3389/fevo.2020.518954

Biedermann, P.H.W., Klepzig, K.D. & Taborsky, M. (2011) Costs of delayed dispersal and alloparental care in the fungus-cultivating ambrosia beetle *Xyleborus affinis* Eichhoff (Scolytinae: Curculionidae). *Behavioral Ecology and Sociobiology*, 65(9), 1753–1761. Available from: https://doi.org/10.1007/s00265-011-1183-5

Biedermann, P.H.W. & Taborsky, M. (2011) Larval helpers and age polyethism in ambrosia beetles. *Proceedings of the National Academy of Sciences*, 108(41), 17064–17069. Available from: https://doi.org/10.1073/pnas.1107758108

Birthwright, A.-T. (2016) Liquid gold or poverty in a cup? The vulnerability of Blue Mountain and High Mountain coffee farmers in Jamaica to the effects of climate change. In: Thomas-Hope, E. (Ed.) *In climate change and food security: Africa and the Caribbean*, 1st edition. London: Routledge Taylor & Francis Ltd., pp. 70–83.

Birthwright, A.-T. & Barker, D. (2015) Double exposure and coffee farming: a case study of the vulnerability and livelihood experiences among small farmers in Frankfield, Jamaica. *Caribbean Geography*, 20, 41–59.

Cooling, M., Hartley, S., Sim, D.A. & Lester, P.J. (2011) The widespread collapse of an invasive species: Argentine ants (*Linepithema humile*) in New Zealand. *Biology Letters*, 8(3), 430–433. Available from: https://doi.org/10.1098/rsbl.2011.1014

Damon, A. (2000) A review of the biology and control of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Bulletin of Entomological Research*, 90(6), 453–465. Available from: https://doi.org/10.1017/S0007485300000584

Dufour, B.P., Kerana, I.W. & Ribeyre, F. (2021) Population dynamics of *Hypothenemus hampei* (Ferrari) according to the phenology of *Coffea arabica* L. in equatorial conditions of North Sumatra. *Crop Protection*, 146, 105639. Available from: https://doi.org/10.1016/j.cropro.2021.105639

Gauthier, N. (2010) Multiple cryptic genetic units in *Hypothenemus hampei* (Coleoptera: Scolytinae): evidence from microsatellite and mitochondrial DNA sequence data. *Biological Journal of the Linnean Society*, 101(1), 113–129. Available from: https://doi.org/10.1111/j.1095-8312.2010.01483.x

Gottlieb, D., Bouskila, A., Sitkov-Sharon, G., Lubin, Y., Harari, A.R. & Taylor, P. (2010) Female palm-seed borer beetles adjust their sex ratio according to relatedness of female neighbours. *Evolutionary Ecology Research*, 12(7), 885–896.

Gottlieb, D., Lubin, Y. & Harari, A.R. (2014) The effect of female mating status on male offspring traits. *Behavioral Ecology and Sociobiology*, 68(5), 701–710. Available from: https://doi.org/10.1007/s00265-014-1683-1

Gugliuzzo, A., Kreuzwieser, J., Ranger, C.M., Tropea Garzia, G., Biondi, A. & Biedermann, P.H.W. (2023) Volatiles of fungal cultivars act as cues for host-selection in the fungus-farming ambrosia beetle *Xylosandrus germanus*. *Frontiers in Microbiology*, 14, 1151078 [Accessed July 5th 2023] Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1151078

Guido, Z., Knudson, C., Finan, T., Madajewicz, M. & Rhiney, K. (2020) Shocks and cherries: the production of vulnerability among smallholder coffee farmers in Jamaica. *World Development*, 132, 104979. Available from: https://doi.org/10.1016/j.worlddev.2020.104979

Hamilton, W.D. (1967) Extraordinary sex ratios. *Science*, 156(3774), 477–488. Available from: https://doi.org/10.1126/science.156.3774.477

Infante, F. (2018) Pest management strategies against the coffee berry borer (Coleoptera: Curculionidae: Scolytinae). *Journal of Agricultural and Food Chemistry*, 66(21), 5275–5280. Available from: https://doi.org/10.1021/acs.jafc.7b04875

Infante, F., Jaramillo, J., Castillo, A. & Vega, F. (2009) The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae): a short review, with recent findings and future research directions. *Terrestrial Arthropod Reviews*, 2(2), 129–147. Available from: https://doi.org/10.1163/187498209X12525675906031

Jaramillo, J., Chabi-Olaye, A., Kamonjo, C., Jaramillo, A., Vega, F.E., Poehling, H.-M. et al. (2009) Thermal tolerance of the coffee berry borer *Hypothenemus hampei*: predictions of climate change impact on a tropical insect pest. *PLoS One*, 4(8), e6487. Available from: https://doi.org/10.1371/journal.pone.0006487

Johnson, M.A., Ruiz-Diaz, C.P., Manoukis, N.C., Carlos, J. & Rodrigues, V. (2020) Coffee berry borer (*Hypothenemus hampei*), a global pest of coffee: perspectives from historical and recent invasions, and future priorities. *Insects*, 11, 882. Available from: https://doi.org/10.3390/insects11120882

Jordal, B.H., Sequeira, A.S. & Cognato, A.I. (2011) The age and phylogeny of wood boring weevils and the origin of subsociality. *Molecular Phylogenetics and Evolution*, 59(3), 708–724. Available from: https://doi.org/10.1016/j.ympev.2011.03.016

Kasson, M.T., Wickert, K.L., Stauder, C.M., Macias, A.M., Berger, M.C., Simmons, D.R. et al. (2016) Mutualism with aggressive wood-degrading *Flavodon ambrosius* (Polyporales) facilitates niche expansion and communal social structure in *Ambrosiophilus* ambrosia beetles. *Fungal Ecology*, 23, 86–96. Available from: https://doi.org/10.1016/j.funeco.2016.07.002

Khanday, A.L. & Buhroo, A.A. (2020) Life cycle and biology of *Pityogenes scitus* Blandford, 1893 (Coleoptera: Curculionidae: Scolytinae), a pest of *Pinus wallichiana* in Kashmir, India. *Journal of Forest Science*, 66(8), 318–328. Available from: https://doi.org/10.17221/8/2020-JFS

Kiran, R. & Venkatesha, M. (2020) Impact of multiple infestations by colonizing coffee berry borer, *Hypothenemus hampei*. *Research Journal of Agricultural Sciences*, 11(2), 434–437.

Mariño, Y.A., Bayman, P. & Sabat, A.M. (2021) Demography and perturbation analyses of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): implications for management. *PLoS One*, 16(12), e0260499. Available from: https://doi.org/10.1371/journal.pone.0260499

Myrie, A., Hall, T., Luke, D., Chinthapalli, B.R., Tennant, P. & Robinson, D. (2023) Coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae): activity and infestation in the High Mountain and Blue Mountain regions of Jamaica. *Insects*, 14(8), 694. Available from: https://doi.org/10.3390/insects14080694

Njihia, T.N., Jaramillo, J., Murungi, L., Mwenda, D., Orindi, B., Poehling, H.M. et al. (2014) Spiroacetals in the colonization behaviour of the coffee berry borer: a 'push-pull' system. *PLoS One*, 9(11), e111316. Available from: https://doi.org/10.1371/journal.pone.0111316

Oliveira, C.M., Auad, A.M., Mendes, S.M. & Frizzas, M.R. (2013) Economic impact of exotic insect pests in Brazilian agriculture. *Journal of Applied Entomology*, 137(1–2), 1–15. Available from: https://doi.org/10.1111/jen.12018

CONSEQUENCES TO CO-HABITATION IN CBBS

Agricultural and Forest
Entomology

Royal
Entomological
Society

11

Pereira, A.E., Gontijo, P.C., Fantine, A.K., Tinoco, R.S., Ellersieck, M.R., Carvalho, G.A. et al. (2021) Emergence and infestation level of *Hypothenemus hampei* (Coleoptera: Curculionidae) on coffee berries on the plant or on the ground during the post-harvest period in Brazil. *Journal of Insect Science*, 21(2), 10. Available from: https://doi.org/10.1093/jisesa/ieab022

Perez, M., Domínguez-López, I., López-Yerena, A. & Vallverdú, Q.A. (2023) Current strategies to guarantee the authenticity of coffee. *Critical Reviews in Food Science and Nutrition*, 63(4), 539–554. Available from: https://doi.org/10.1080/10408398.2021.1951651

Raffa, K.F. (2001) Mixed messages across multiple trophic levels: the ecology of bark beetle chemical communication systems. *Chemoecology*, 11(2), 49–65. Available from: https://doi.org/10.1007/PL00001833

Rhodes, L.F. & Mansingh, A. (1986) Distribution of the coffee berry borer *Hypothenemus hampei* Ferr. in Jamaica, and an assessment of the chemical control programme (1979–1982). *International Journal of Tropical Insect Science*, 7(4), 505–510. Available from: https://doi.org/10.1017/S1742758400009735

Statistical Institute of Jamaica. (2018) International Merchandise Trade Statistical Bulletin January to December 2018. Kingston, Jamaica. Available from: https://statinja.gov.jm/PublicationReleases.aspx

Vega, F.E. (2008) Coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae). In: Capinera, J.L. (Ed.) *Encyclopedia of entomology*. Dordrecht, the Netherlands: Springer Netherlands, pp. 959–960. [Accessed June 8th 2023]. Available from: https://doi.org/10.1007/978-1-4020-6359-6_750

Vega, F.E., Franqui, R.A. & Benavides, P. (2002) The presence of the coffee berry borer, *Hypothenemus hampei*, in Puerto Rico: fact or fiction? *Journal of Insect Science*, 2, 13.

Vega, F.E., Kramer, M. & Jaramillo, J. (2011) Increasing coffee berry borer (Coleoptera: Curculionidae: Scolytinae) female density in artificial diet decreases fecundity. *Journal of Economic Entomology*, 104(1), 87–93. Available from: https://doi.org/10.1603/ec10353

Vega, F.E., Simpkins, A., Bauchan, G., Infante, F., Kramer, M. & Land, M.F. (2014) On the eyes of male coffee berry borers as rudimentary organs. *PLoS ONE*, 9(1), e85860. Available from: https://doi.org/10.1371/journal.pone.0085860

Vega, F.E., Simpkins, A., Rodríguez-Soto, M.M., Infante, F. & Biedermann, P.H.W. (2017) Artificial diet sandwich reveals subsocial behaviour in the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae). *Journal of Applied Entomology*, 141(6), 470–476. Available from: https://doi.org/10.1111/JEN.12362

Vilchez-Mendoza, S., Romero-Gurdián, A., Avelino, J., DeClerck, F., Bommel, P., Betbeder, J. et al. (2022) Assessing the joint effects of landscape, farm features and crop management practices on berry damage in coffee plantations. *Agriculture, Ecosystems and Environment*, 330, 107903. Available from: https://doi.org/10.1016/j.agee.2022.107903

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. The frequency of interactive behaviours varies with time.

Table S2. The frequency of interactive behaviours varies between treatments.

Table S3. The frequency of individual interactive behaviours varies with time.

Table S4. The frequency of individual interactive behaviours varies between treatments.

Table S5. Correlation between the frequency of each behaviour and total offspring numbers with respective *p*-values.

Figure S1. Per capita production of eggs, larvae, pupae and young adults by single beetles and pairs of beetles. The number of reproducing females affected the number of larvae and pupae produced per capita, but not the number of eggs or young adults (eggs: t-test, $t = 0.521$, $p > 0.604$; larvae: t-test, $t = 2.234$, $p = 0.029$; pupae: t-test, $t = 2.308$, $p = 0.024$; young adults: t-test, $t = 0.661$, $p = 0.512$). Boxplots show medians and upper and lower quartiles.

Figure S2. The frequency and duration of all behaviours are positively correlated.

Picture S1. Single-infested berries. (A) A mature red berry already occupied by a CBB, and (B) and (C) show a single female CBB boring through the pinhole of a coffee berry at the mature ripe berry stage of development (red berry).

Picture S2. Double-infested coffee berry at the mature ripe berry stage (Whitney Cranshaw, Colorado State University, Bugwood.org).

Picture S3. (A) Artificial diet sandwiches used to assess productivity, sex ratio and behaviour of the CBBs. (B) Initial setup of artificial diet representing single (one female) and double-infested berries (two females).