Sun vs. shade affects infestation, total population and sex ratio of the coffee berry borer (*Hypothenemus hampei*) in Puerto Rico

Yobana A. Mariño^{a*}, Maria-Eglée Perez^b, Fernando Gallardo^c, Marella Trifilio^a, Michelle Cruz^a and Paul Bayman^a.

^a**Department of Biology, University of Puerto Rico – Río Piedras, PO Box 23360, San Juan, PR 00931-3360, USA.**

^b**Department of Mathematics, University of Puerto – Río Piedras, PO Box 23355, San Juan, PR 00931-3355, USA.**

^c**Department of Crops and Agroenvironmental Sciences, University of Puerto Rico – Mayagüez, PO Box 9000, Mayagüez, PR 00681, USA.**

*** Corresponding author: Tel: +1-787-466-1941; fax: +1-787-764-3875**

E-mail addresses: yandreamarinoc@gmail.com; yandreabiologa@yahoo.com

Postal Address: Departamento de Biología, Universidad de Puerto Rico – Río Piedras, PO Box 23360, San Juan, PR 00931-3360, USA

Abstract

Sun and shade coffee (*Coffea arabica* L.) provide different biotic and abiotic environments which can affect the damage, distribution and reproduction of coffee pests. However, the effect of shade on damage by the coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari), the most important coffee pest worldwide, is controversial and unclear. We compared infestation, total population per fruit and sex ratio of the CBB between shade and sun coffee, during two coffee-growing seasons in Adjuntas, Puerto Rico. According to generalized linear mixed models, CBB infestation

was significantly higher in shade plots, and ranged from 7 - 52% compared with 4 - 26% in sun. However, the total population per fruit was higher in sun coffee fruits than in shade coffee, and the sex ratio was more biased to females in sun coffee. Sun coffee was characterized by higher temperatures and lower relative humidity. Environmental variables appeared to be important drivers of CBB population densities in field, with positive correlations between temperature and the number of pupae, juveniles, females and males. This tendency of larger populations in sun plots could represent a serious threat for coffee. In addition to fewer CBB in infested fruits in shade plots, we observed that shade coffee also had more natural enemies of CBB such as ants and entomopathogenic fungi.

Keywords: Coffee berry borer; coffee agroecosystem; microclimate; pest; life cycle

Corresponding author: Yobana A. Mariño-Cárdenas

Phone number: (1) 787-466-1941

Fax number: (1) 787-764-3875

e-mail: yandreamarinoc@gmail.com

1. Introduction

The coffee berry borer (CBB) *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae) is the most devastating pest of coffee worldwide (Damon, 2000; Soto-Pinto *et al.*, 2002; Jaramillo *et al.*, 2006; Vega *et al.*, 2009). The CBB depends principally on coffee fruits for food, reproduction, and development (Damon, 2000). Economic losses are consequences of: (1) Drilled young fruits that abscise and fall to the ground, (2) Drilled green and red fruits that do not abscise, but because of CBB damage have low weight and quality at harvest (Damon, 2000; Webge *et al.*, 2003).

In the last few decades, intensification of coffee production has changed the coffee agroecosystem. Coffee (*Coffea arabica* L.) evolved in Ethiopian forests as an understory tree, and is thus shade-adapted (Soto-Pinto *et al.*, 2002; DaMatta *et al.*, 2007). When coffee was introduced to Asia and Latin America, initially it was grown under shade to retain the physiological attributes of shade plants. In the 1980s the use of shade was abandoned or reduced to increase production (Perfecto *et al.*, 1996; DaMatta and Rodríguez, 2007) and to combat fungal diseases, specifically the coffee leaf rust *Hemileia vastratix* (Perfecto *et al.*, 1996; DaMatta *et al.*, 2002; Soto-Pinto *et al.*, 2002) and pests, especially CBB (Beer *et al.*, 1998; DaMatta *et al.*, 2002). This modernization changed abiotic characteristics such as temperature and relative humidity (Teodoro *et al.*, 2008), biotic characteristics such as crop density, presence of herbivores, natural

enemies of CBB (e.g. ants and pathogens, (Letourneau *et al.*, 2009), and resource quality and quantity (Hunter and Price, 1992). These changes affected directly and indirectly the CBB's abundance, survival and reproduction (Bale *et al.*, 2002; Silva *et al.*, 2011).

Several studies have investigated the effect of coffee agroecosystem (i.e., sun vs. shade) on infestation of CBB, but the conclusions are unclear. Some authors have reported that shade increases the infestation of CBB (Wrigley, 1988; Bosselmann *et al.* 2009; Larsen and Philpott, 2010); others argued that the effect of shade on CBB infestation is also related to differences in shade levels, canopy tree species (Féliz *et al.* 2004) and coffee management (Sánchez *et al.* 2013). In contrast, others have found more CBB infestation in sun coffee (Jaramillo *et al.* 2013) or no significant effect (Soto-Pinto *et al.* 2002).

Even less clear is the effect of sun vs. shade on population sizes and sex ratio of CBB. Recently it has been argued that the CBB is heat-tolerant and population growth increases with temperature (Jaramillo *et al.*, 2009; Burgiel and Muir, 2010). Modeling and climatic data suggest the CBB is favored by rising temperatures as a consequence of climate change, increasing its distribution and damage to coffee (Jaramillo *et al.*, 2011). These diverse results may partly reflect the fact that the studies have been done in diverse countries, with varying conditions.

The objective of this study was to compare coffee planted under shade vs. sun in terms of CBB infestation, total population and sex ratio over the course of the coffee-growing seasons of 2010 and 2011 in Puerto Rico. We asked the following questions:

- 1) Does the coffee agroecosystem (shade vs. sun) affect the infestation rate of CBB? We predicted that CBB would attain higher infestation in shade coffee than in

sun coffee as a consequence of more favorable temperatures, less drastic fluctuations in daily temperature, higher humidity and protection from the wind.

2) Does sun vs. shade coffee affect the population size and sex ratio of CBB within infested fruits? We hypothesized that fruits exposed to direct sunlight and higher temperatures will have larger CBB populations and more biased sex ratios (higher female: male) per fruit because more resources are available.

2. Materials and Methods

2.1. Study region and site descriptions

The study was carried out in three private farms in Adjuntas, F1 (18°10'42.5"N, 66°44'36.3"W), F2 (18°10'45.5"N, 66°46'23.6"W) and F3 (17°57'4.5"N, 66°23'15"W). Adjuntas is part of the coffee-growing region in the central mountains of Puerto Rico and has an altitude of ~550 m above sea level, temperatures of 15-28°C and mean annual precipitation of ~1870 mm (Harmsen *et al.*, 2009). In each farm two plots were chosen: 1) sun coffee (managed as a monoculture and exposed to full sunlight), and 2) shade coffee (with shade provided by up to nine tree species) (Table A.1). During 2010, we sampled farms F1 and F2, but in 2011 we had to replace farm F2 with F3, because in F2 the grower had pruned all sun coffee plants.

The management of the three farms was similar: glyphosate was applied 3-4 x per year to control weeds. No chemicals were applied to control the CBB; however, on farms F1 and F3 the growers applied the entomopathogenic fungus *Beauveria bassiana* (commercial name Mycotrol[®]), only one time in the year previous to our sampling. Also, the three growers conducted a rigorous collection of all fruits in the plant after harvest to remove reservoirs of CBB between crops (a technique called 're-re' in Spanish) (Benavides *et al.*, 2003; Jaramillo *et al.*, 2006).

From September 2011 to February 2012, temperature and relative humidity were measured using Onset HOBO[®] H8 Pro Series data loggers (data logger H08-32-08 and humidity sensor HUM-UPS-500, Onset Computer Corporation, Bourne, MA, USA). Three data loggers were placed in the center of each plot; these were hung on a coffee plant at 1.5 m above the ground. Measurements were taken every minute and the 30-min average was logged automatically. Canopy cover was measured by photographs with a Canon EOS 100D equipped with a 4.5 mm F2.8 EXDC circular fisheye lens. The camera was mounted with the top north on a tripod at 100 cm above the ground. Photographs were taken in the morning (10:00 – 12:00 Atlantic Standard Time). Ten photographs were taken in each plot and analyzed using Gap Light Analyzer (GLA Version 2.0, Frazer *et al.*, 1999). GLA gives the percentage of canopy openness; we used these values to determine canopy cover of shade plots as 1 – proportion of canopy openness.

2.2. Sampling methods

Infestation of CBB was surveyed from June to November 2010 and 2011. This period included 120 DAF (days after main flowering), harvest and approximately 30 days after harvest. Twenty coffee plants were randomly selected in each plot and the central branch was tagged; the number of fruits and CBB-bored fruits on this branch was counted, in the first assessment, bored berries were tagged. The same branches were censused every two weeks, and newly-bored fruits were recorded and marked.

Population sizes were surveyed from July to December; this was a month longer than the sampling for infestation, extended to evaluate the population in fruits that remained on the plants after the main harvest. Every two weeks three bored fruits were collected from ten tagged plants, giving 120 fruits per sampling date for a total of 2,640.

The fruits were removed from different branches than those that were tagged for the evaluation of CBB infestation. These coffee fruits were dissected with the aid of a dissecting microscope at 30-50X; we counted the number of all developmental stages of CBB (i.e., eggs, larvae, pupae, juveniles and adults). In 2011 the fruits collected from September to December were classified into three stages of maturity (green, yellow and red). All adults and juveniles were sexed, and the sex ratio was expressed as the proportion of females in the population of juveniles plus adults.

The position of colonizing females inside the fruit was classified following Bustillo *et al.* (1998): (A) when the colonizing female has started to drill the fruit but has not yet reached the endocarp, and is not present; (B) when the female has penetrated the endocarp, but not the endosperm; (C) when the female has reached the endosperm but has not oviposited yet; (D) when the female has made her gallery in the endosperm, and immature stages of the CBB are found.

Artesanal traps were used to monitor CBB females flying in search of new fruits from July 2010 to March 2012. We placed six traps per plot, four at the ends and two in the center; all traps were hung 1.5 m above the ground. Traps were made from 2 L plastic soft drink bottles, the outer surface painted red with five windows (12 X 3.0 cm) cut 10 cm above the bottom (Uemura-Lima *et al.*, 2010). A mix of ethanol-methanol (1:1) as an attractant was placed in 50 mL plastic centrifuge tubes, and these were hung from a wire inside the bottle 8 cm below the top. The bottom of the bottle contained 200 mL of water with 1% liquid detergent, added to trap CBB females. The mix of water and detergent was replaced monthly, and CBB were counted by direct counts or by weight, depending on the density of capture.

2.3. Statistical analysis

For statistical analysis we considered as response variables the following CBB descriptors: infestation of CBB, total population per fruit, position of colonizing females and sex ratio. The explanatory variables were: type of coffee (sun vs. shade), year (2010 and 2011), farms (F1, F2 and F3) and sampling dates.

One-way ANOVAs were performed to determine significance of differences in daily maximum, minimum temperature and the daily temperature fluctuations; mean and minimum relative humidity and percentage of canopy openness in sun vs. shade, and canopy cover between shade plots.

Data from infestation and total population per fruit were analyzed separately using generalized linear mixed models (GLMM) with binomial and Poisson error distributions respectively. The variables type of coffee (sun vs. shade) and sampling date were included as fixed factors; year and farm as random factors. For analysis of data from infestation we considered the sampling dates as repeated measures for each branch.

Positions of the colonizing female inside the coffee berry (A, B, C and D) and females caught with traps in sun vs. shade were analyzed using a generalized linear model (GLM) with a binomial and Poisson errors distribution respectively.

To evaluate CBB sex ratio, we used only the data from fruits in which we observed juveniles and adults (the CBB stages where individuals can be sexed by visual inspection). We calculated the sex ratio for each fruit as the proportion of CBBs that were females, counting only fruits with males, because the reproduction of the CBB has been described as exclusively sexual (Barrera *et al.*, 1995; Gingerich *et al.*, 1996; Constantino *et al.*, 2011). ANOVAs followed by post-hoc Tukey tests were used to test differences in the sex ratio in sun vs. shade; the data were transformed to $\arcsin(x)$ to fulfill assumptions of a normal distribution.

Linear regressions were used to determine the relationship between number of individuals in different stages of development and sex ratio with daily temperature fluctuation, maximum, minimum, median and mean temperature (°C) and minimum and mean relative humidity (%), based on biweekly averages of measurements previous to the sampling. All data analyses were performed in R and GLMM models were fitted using the R package lme4 (R core Team, 2013).

3. Results

3.1. Environmental characteristics of plots

Sun vs. shade coffee differed significantly in all temperature and humidity variables evaluated (Table 1). Sun coffee plots were characterized by higher maximum temperatures and lower relative humidity (38.2 °C and 26.7% respectively) compared with shade coffee plots (30.7 C° and 57.7%).

The GLA results showed that sun plots differed significantly from shade plots in canopy openness (69% in sun, 17% in shade; $F=431.6$, $df=1$, $p < 0.001$). No differences were observed in % canopy cover among shade plots: 86% for F1, 79% for F2 and 84% for F3 ($F = 2.10$, $df=2$, $p = 0.1420$).

3.2. Effect of sun vs. shade coffee on infestation of CBB

The estimated probability of infestation was significantly greater in shade than in sun: infestation in 2010 and 2011 were respectively 7 - 52% in shade and 4 - 26% in sun (Table 2 and 3). The sampling date also had a significant effect on the probability of CBB infestation (Table 2); percentages of infestation increased through time and were highest in the last sampling in November for both sun and shade (Fig. 1a).

3.3. Effect of sun vs. shade coffee on total number of CBB per fruit

Contrary to infestation, the estimated abundance of individuals was significantly higher in sun than shade coffee (Table 2 and 3). The sampling date also had a significant effect on CBB abundance (Table 2); in both sun and shade, the percent of bored fruits with reproduction and the number of CBB per bored fruit increased significantly through the coffee-growing season. In sun plots, largest populations were observed in October, whereas in shade, where fruits develop more slowly, the peak was observed in November (Fig. 1b).

When each life cycle stage was considered separately, the estimated abundances of eggs, pupae, juveniles and adults per bored coffee fruit were significantly higher in sun plots (GLMM, poisson errors, eggs $Z = 4.21$, $p < 0.0001$; pupae $Z = 6.69$, $p < 0.0001$, juveniles $Z = 6.31$, $p < 0.0001$ and adults $Z = 6.76$, $p < 0.0001$). However, the abundance of larvae did not differ significantly between sun and shade (larvae, $Z = 0.99$, $p = 0.32$) (Fig. 2). Our sampling strategy did not permit detection of adult progeny that had already left the fruits.

3.4. Environmental variables and relationship to number of CBB per bored fruit

In sun plots, the total number of some stages was positively and significantly correlated with mean temperature (pupae: $p = 0.002$, $R^2 = 0.48$; juveniles: $p = 0.039$, $R^2 = 0.40$; females: $p = 0.031$, $R^2 = 0.43$; males: $p = 0.037$, $R^2 = 0.47$) and the median temperature (females: $p = 0.037$, $R^2 = 0.41$). In shade plots, the total number of juveniles was positively correlated with minimum temperature ($p = 0.03$, $R^2 = 0.25$), mean temperature ($p = 0.007$, $R^2 = 0.39$) and median temperature ($p = 0.001$, $R^2 = 0.54$); pupae with daily temperature fluctuation ($p = 0.01$, $R^2 = 0.34$) and females with the median

temperature ($p=0.031$, $R^2 = 0.26$). No stage of development of CBB was correlated with relative humidity in either sun or shade.

3.5. Effect of sun vs. shade coffee on positions of colonizing females of *H. hampei*

The proportions of colonizing *H. hampei* females in the four positions inside the fruits as a function of sun vs. shade are presented in Fig. 3. The proportion of colonizing *H. hampei* females in each position varied during the season; position A was most frequent in June (28%), when penetration was just starting, followed by July (20%) and August (20%). Position B was most frequent in June (64%) and July (52%) and position C in July (25%) and August (28%). Position D was most frequent in November (61%) and December (53%), when fruits were ripest, followed by October (51%) and September (45%).

Only the number of females in positions B and D were affected by the type of coffee (GLM, binomial errors, position B: $Z=4.60$, $p<0.001$; position D: $Z=4.64$, $p<0.001$). Position B were was more frequent in shade (33%) than in sun (25%); whereas position D was higher in sun (43%) than in shade (34%) (Fig 3a); in other words, more colonizing females reached the endosperm and reproduced in sun than in shade.

3.6. Differences between sun vs. shade on trap capture rates

The estimated abundance of females caught in traps varied significantly between sun vs. shade coffee and during the growing season (glm, Poisson errors, sun vs. shade $\chi^2_1 = 3794$, $Z=61.66$, $P<0.0001$; months $\chi^2_{10} = 661479$, $Z=778.02$, $P<0.0001$). Capture was significantly higher in sun than shade coffee. Highest capture rates were obtained from February to June and November and December with a marked peak in March, when fewer coffee fruits were available for attack (Fig 4).

3.7. Effect of sun vs. shade coffee on CBB sex ratio

We found significant differences in CBB sex ratio in sun vs. shade coffee ($F=4.47$, $df=1$, $p = 0.03$); the sex ratio was more skewed towards females in sun than shade plots (Table 3). No males were found in 77.5% of shade fruits and 80.1% of sun fruits (counting only fruits with progeny of juveniles and adults) but this difference was not significant ($F= 0.17$, $df=1$, $p = 0.67$) (Table 3). No correlation was found between CBB sex ratio and any environmental variable.

4. Discussion

This study showed that sun vs. shade coffee has a significant effect on CBB infestation, total population per fruit, and sex ratio. Infestation of CBB was significantly greater in shade coffee, while CBB individuals per bored fruit were higher in sun coffee, and the sex ratio was more biased to females in sun coffee.

Overall, the infestation of CBB in Adjuntas, Puerto Rico in this study was high, ranging from 4.5 - 52% of fruits surveyed (Table 3). This infestation is higher than in previous studies: for example, in Brazil in 1992-'93 infestation was 21 - 32% (Cure *et al.*, 1998); in Mexico in 1997 infestation was 0.1 - 19% (Soto-Pinto *et al.*, 2002), and 5-35% in 2008 (Larsen and Philpott, 2010). In Colombia infestation was <2 - 25% in 1995 and < 2 - 13% in 1996 (Benavides *et al.*, 2003). Perhaps the most relevant comparison is with Mexico in 1983, about five years after CBB was first reported there, when infestation was 10-15% (Barrera, 2005); CBB arrived in Puerto Rico in 2007 (NAPPO, 2007); four years later, in our second sampling season, far more fruits were infested than in any of the above-cited studies. CBB is considered the most important pest of

coffee worldwide, but if the study sites in Adjuntas are representative, the level of damage in Puerto Rico appears to be unprecedented.

The majority of the coffee plantations in Puerto Rico can be considered untreated, because many growers are not using the cultural and biological tools available for CBB control. For example, many growers do not use the artesanal traps and the lack of pickers does not allow a rigorous collection of all fruits that remain on the plant and the ground after harvest to remove reservoirs of CBB between crops (Benavides *et al.*, 2003; Jaramillo *et al.*, 2006).

4.1. Effect of sun vs. shade coffee on infestation of CBB

CBB infestation was significantly higher in shade than in sun: from 7- 52% in shade vs. 4 - 26% in sun (Table 3). Previous studies also found that shade favored infestation (Wrigley, 1988; Bosselmann *et al.* 2009; Larsen and Philpott, 2010). Several studies have shown that the use of shade trees over coffee plots influences abiotic factors such as temperature, humidity and wind (Barradas *et al.* 1982; Siles *et al.* 2010; Jaramillo *et al.* 2013). Our shade plots had higher humidity levels, lower maximum temperatures and more stable daily temperature fluctuations than the sun plots (Table 1); these environmental conditions are conducive to CBB damage (Damon, 2000; Vega *et al.* 2009).

4.2. Effect of sun vs. shade coffee on total number of CBB per fruit

Relatively few studies have compared number of CBB per infested fruit between sun and shade coffee. Here, contrary to Sanchez *et al.* (2013) and Féliz *et al.* (2004), we found significantly higher populations of CBB in sun than in shade fruits. This contradiction may be due to differences in percentages of shade between their shade

plots and our plots, for example, shade plots evaluated by Féliz *et al.* (2004) had a 60 – 70% of canopy cover and our plots had a cover over 79%; differences in degree of shade can affect abiotic factors such as temperature. The insects' reproduction can be influenced by biotic and abiotic factors (Bale *et al.*, 2002; Silva *et al.*, 2011). Among abiotic factors, several studies have demonstrated a significant correlation between temperature and CBB reproduction: Jaramillo *et al.* (2009) reared the CBB in the laboratory at eight temperatures from 15 to 35 °C and found that development was faster and the finite rate of increase (λ) was greater at higher temperatures. Field experiments also showed a positive correlation between temperature and CBB population sizes (Teodoro *et al.*, 2008). Also, Teodoro *et al.* (2009) observed higher populations in 'intensively managed' plots, which like our sun plots had higher temperatures and lower relative humidity than shade (Table 1).

Although we did not find a significant correlation between temperature and total population, as Teodoro *et al.* (2008) did, we found that the numbers of individuals of some stages of development were correlated with temperature; in both sun and shade coffee, the number of females and juveniles was positively correlated with the mean and median temperature. The number of juveniles was also positively correlated with minimum temperature in shade plots and the number of males and pupae were positively correlated with mean temperature in sun plots. Contrary to Teodoro *et al.* (2009) we did not observe any influence of relative humidity on CBB populations.

CBB populations can also be influenced by biotic factors such as natural enemies. Shade plots with higher plant diversity and structural complexity have higher diversity and abundance of natural enemies of CBB: parasitoids, ants, pathogens and birds (Perfecto *et al.*, 1996; Greenberg *et al.*, 1997; Armbrrecht and Gallego, 2007; Philpott *et al.*, 2008; Letourneau *et al.*, 2009; Johnson *et al.*, 2010; Larsen and Philpott,

2010). This higher abundance and diversity of natural enemies in shade may partly explain our higher population densities of CBB in sun coffee, and the higher percentage of fruits that were bored but with no reproduction inside in shade plots.

Armbrecht and Gallego (2007) found that shade coffee in Colombia is more diverse in species of ants than sun coffee; also, CBB adults put into spiral traps were more frequently removed from the traps installed in shade coffee than sun coffee. The ant genus *Solenopsis* was the most abundant in both sun and shade. Species of *Solenopsis* and *Azteca* are predators of CBB, and small *Solenopsis* can penetrate infested fruits through CBB entry holes and may remove CBB females and their progeny (Armbrecht and Perfecto, 2003; Armbrecht and Gallego, 2007; Pardee and Philpott, 2011). We occasionally observed larvae, pupae and adults of *Myrmelachista* ants inside the fruits, and in these fruits we did not observe any CBB individuals; these ants may penetrate the fruits to predate the borers and/or use the fruits to reproduce. Like Armbrecht and Gallego (2007) we found *Myrmelachista* only in shade coffee.

Another important natural enemy of CBB is the fungus *Beauveria bassiana* (De la Rosa *et al.*, 1997; Haraprasad *et al.*, 2001; Vega *et al.*, 2008). We observed that in shade plot F3 CBB-infested fruits with *Beauveria* were common, and this plot had high CBB infestation but low reproduction. In shade coffee microbial insecticides are more effective than in sun coffee; in sun plots direct sunlight, higher temperatures and lower humidity impede fungal germination, establishment and survival (Inglis *et al.*, 2001; Pucheta *et al.*, 2006). Fruits from sun plots are more exposed to direct sunlight and the spores' photoinactivation by UV has been described as the most limiting environmental factor (Edgington *et al.*, 2000).

These tendencies of higher infestation in shade and higher total population in sun were maintained for both years of sampling (Table 3). However, infestation was

higher in 2011 than 2010. Previous studies suggested a significant effect of rainfall on CBB dynamics (Constantino *et al.*, 2010; Constantino *et al.*, 2011a; Rodríguez *et al.*, 2013). CBB infestation was higher (> 40%) during a dry year in Colombia, while in a wetter year it was 1 – 12 % (Constantino *et al.* 2011a). Our results agree with their observations: 2010 was wetter than 2011; cumulative rainfall was 3,096 mm in 2010 and 2,279 mm in 2011, compared with the annual historic average from 1981 – 2010 of 1,972 mm (SERCC, 2007). Rainfall also affects the survival of adult and immature CBB in remaining fruits after harvest. Higher rainfall induces faster decomposition of the fruits and enhances the proliferation of fungi and bacteria which can infect CBB (Baker *et al.*, 1992; Damon, 2000; Ruiz-Cárdenas and Baker, 2010; Rodríguez *et al.*, 2013).

4.3. Coffee fruit development and its relation with CBB infestation and total population per fruit

In both sun and shade coffee the infestation and total population of CBB per fruit increased during the season. Several studies have reported a close relation between coffee fruit maturity and CBB damage (Cure *et al.*, 1998; Mathieu *et al.*, 1999; Damon, 2000; Camilo *et al.*, 2003; Valdés and Ravelo, 2006; Teodoro *et al.*, 2009; Vázquez *et al.*, 2012). However, total population peaked in October in sun coffee and November in shade coffee (Fig. 1b); this difference in time is a consequence of the delay of approximately one month in the maturation of coffee fruits in plots with high levels of shade (Vaast *et al.* 2006; Geromel *et al.*, 2008).

Independently of type of coffee (sun vs. shade), we found an increase in total CBB as fruits matured (Table 4). Several researchers have shown a direct relation

between fruit ripening and CBB densities (Cure *et al.* 1998, Camilo *et al.* 2003, Mathieu *et al.*, 1999).

Our results of trap collection also showed a relation between coffee crop phenology and capture densities; this has been demonstrated in previous studies (Mathieu *et al.*, 1999; Barrera *et al.*, 2006; Vázquez *et al.*, 2012). The capture density increased after the main harvest, at which time the availability of coffee fruits on the trees declines rapidly. We also observed a pronounced peak in March with a capture density of 1700 to 7400 females per trap; these captures correspond to the period from mid-February to mid- March (Fig. 4). March had high rainfall and humidity, which stimulate females' emergence and dispersion (Baker *et al.*, 1992; Mathieu *et al.*, 1999; Damon, 2000; Barrera *et al.*, 2006).

Our results show that artisanal traps are an efficient method for management of the CBB. The traps were successful at catching CBB females; a total of 375,000 were caught in 36 traps over 20 months for F1, and 11 months for the farms F1 and F2.

4.4. Effect of sun vs. shade coffee on positions of colonizing females of H. hampei

Damage to coffee fruits was less in shade coffee than sun coffee; we observed that in shade coffee fewer colonizing females reached position D, penetrated the endosperm and reproduced, 34% compared to 43% in sun (Fig. 3a); this means that many shade coffee fruits are drilled but the seed is not damaged. Jaramillo *et al.* (2013) described the same tendency in positions of colonizing females between sun and shade coffee. We agree with their suggestion that differences in chemical composition (Vaast *et al.* 2006) between sun and shade coffee could affect the position of the colonizing females; also, many shade coffee fruits do not have the optimal physical characteristics such as percent dry matter (Alonzo, 1984) for CBB reproduction.

Position of colonizing females can also be affected by temperature: at high temperatures (35°C) in laboratory experiments, females do not reach the endosperm and remain in position B (Jaramillo *et al.*, 2009). However, females from our sun plots were exposed to temperatures up to 38.2 °C and we found a higher proportion of females in position D (43%). Females reared in the laboratory were exposed to constant temperature, whereas in the field temperatures fluctuate. In sun plots, nighttime temperature ranged from 13 - 15 °C, which may have allowed CBB to start the colonization and reach the endosperm; Sponagel (1994, cited by Damon, 2000) suggested females can reach the endosperm in approximately 8 hours.

4.5 Effect of sun vs. shade coffee on CBB sex ratio

We found a significant effect of sun vs. shade on CBB sex ratio, which was more skewed towards females in sun than shade (Table 3). This is the first report that environmental conditions affect CBB sex ratios in the field; in lab experiments CBB sex ratio was not affected by temperatures from 15 to 35 °C (Jaramillo *et al.*, 2009).

Insect sex ratio can be influenced by genetic factors such as sex determination systems (Normark, 2003), extra chromosomal factors including endosymbiotic bacteria (Ebbert and Wrensch, 1993; Weeks *et al.*, 2003; Werren *et al.*, 2008; Kageyama *et al.*, 2012) and environmental factors such as temperature (Nigro *et al.*, 2007; Ross *et al.*, 2011), population densities (Charnov *et al.*, 1981; Kirkendall *et al.*, 1993; King and D'Souza, 2004) and food resources (Rosenfeld and Roberts, 2004; Berndt and Wratten, 2005).

The CBB usually has a sex ratio skewed 10:1 towards females. This deviation has been ascribed to its sex determination system, which is functional haplodiploidy (Brun *et al.*, 1995); female-biased sex ratios are common in haplodiploid organisms

(Vega *et al.*, 2002; Jaramillo *et al.*, 2006). It has also been suggested that extrachromosomal factors, mainly *Wolbachia*, could play a role in the biased sex ratio of CBB; Vega *et al.* (2002) reported the presence of this bacterium in CBB from several countries.

In this study sun coffee was characterized by higher temperatures than shade (Table 1). According to sex allocation theory, a female who is exposed to extreme environmental factors (such as high temperature) should bias her offspring sex ratio towards the sex that suffers less (Ross *et al.*, 2011). Several studies have shown that insects exposed to higher temperatures produce more daughters (Nigro *et al.*, 2007; Ross *et al.*, 2011). In our case, we observed higher number of females per fruit in sun plots (Table 3) and a positive correlation between number of females and mean and median temperature.

In addition to female-biased sex ratios, 61-81% of fruits with CBB progeny did not contain males (Table 3). There are two possible explanations; the first is parthenogenesis. It is assumed that CBB reproduction is exclusively sexual (Barrera *et al.*, 1995; Gingerich *et al.*, 1996; Constantino *et al.*, 2011b); however, Muñoz (1989) found that the CBB can be parthenogenetic, since isolated females in the laboratory produced 55% fertile eggs without fertilization. We suggest that parthenogenesis may occur in the field. Also, in some of these fruits without males, we found eight females or more with other CBB stages: eggs, larvae, pupae. These females might be progeny of the original colonizing female, producing fertile eggs without fecundation.

The second possible explanation is associated with male behavior. According to the literature, males are smaller than females, have reduced, degenerate wings, are incapable of flight and never leave the fruit (Brun *et al.*, 1995; Gingerich *et al.*, 1996; Damon, 2000; Jaramillo *et al.*, 2006); However, it is possible that under certain

conditions males also can leave the fruit after having fertilized their sisters, which would affect observed sex ratios.

5. Conclusions and recommendations

This study provides evidence that the type of coffee agroecosystem can affect the infestation, population density and sex ratio of the coffee berry borer *H. hampei*. We observed higher densities of CBB and a stronger sex ratio skew towards females in sun plots, which were characterized by higher temperatures. Jaramillo *et al.* (2009) recommended the re-introduction of shade trees into sun coffee plantations to mitigate the effect of climate change on coffee plants and the CBB; we observed that in shade coffee the maximum temperature was about 8°C lower and humidity was 20% higher. Moreover, shade coffee fruits with CBB reproduction contained fewer individuals than fruits from sun.

To mitigate the severe damage that CBB is causing in Puerto Rico, which judging by our limited sampling in Adjuntas may be even more severe than in other countries, we recommend the re-introduction of shade trees in sun plantations combined with the use of natural enemies, specifically *Beauveria bassiana*. Our results suggest the best time to apply the fungus is June and July, when the majority of colonizing females are in susceptible positions. Also, our results suggest that artesanal traps are an efficient method for management of the CBB; these traps were most effective from November to June, a period that included post-harvest, interharvest and early fruit growth.

Future research will be focused on elucidating the contribution of biotic factors such as natural enemies on the survival of colonizing females and the reduction of CBB populations. Also, shade levels under 80% percent (the average for our three farms) should be tested in order to determine the optimum level of shade to maintain a balance

between CBB damage control and productivity. An ongoing census is expanding the present study to other parts of the coffee-growing region of Puerto Rico.

Acknowledgments

We are grateful to the growers (Melín Rullán, Anibal and Niulka) for hospitality and permission to conduct experiments on their farms. We thank Victor J. Vega, Fabiola Areces, Luis A. Ramírez, Ana Pamela Torres, Maria Dávila and Virmarie Díaz for their help in fieldwork. We thank the personnel of the UPR Agricultural Experimental Substation in Adjuntas, especially Wigmar González and Alvaro Serrano. We also thank the NSF CREST-CATEC program and its director Elvira Cuevas for support of undergraduate students. We thank Stephen A. Rehner (USDA ARS) for guidance, advice and support. This project was supported by USDA ARS Specific Cooperative Agreement 58-1245-4-083.

References

- Alonzo, P. 1984. El problema de la broca (*Hypothenemus hampei* (Ferr.)) (Col: Scolytidae) y la caficultura. Aspectos relacionados con importancia. Daño, identificación, biología, ecología y control. IICA- PROMECAFE, pp 242.
- Armbrrecht, I., Gallego, M.C., 2007. Testing ant predation on the coffee berry borer in shaded and sun coffee plantations in Colombia. Entomol. Exp. Appl. 124, 261-267.
- Armbrrecht, I., Perfecto, I., 2003. Litter-twig dwelling ant species richness and predation potential within a forest fragment and neighboring coffee plantations of contrasting habitat quality in Mexico. Agric. Ecosyst. Environ. 97, 107-115.
- Baker, P., Barrera, J., Rivas, A., 1992. Life-history studies of the coffee berry borer (*Hypothenemus hampei*, Scolytidae) on coffee trees in southern Mexico. J. Appl. Ecol., 656-662.
- Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J., Good, J.E.G., Harrington, R., Hartley, S., Jones, T.H., Lindroth, R.L., Press, M.C., Symrnioudis, I., Watt, A.D., Whittaker, J.B., 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biol. 8, 1-16.

Barradas, V., Fanjul, L. 1986. Microclimatic characterization of shade and open-grown coffee (*Coffea arabica* L.) plantations in Mexico. Agric. For. Meteorol. 38, 101-112.

Barrera, J., Gómez, J., Alauzet, C., 1995. Can the coffee berry borer (*Hypothenemus hampei*) reproduce by parthenogenesis?. Entomol. Exp. Appl. 77, 351-354.

Barrera, J.F., 2005. Investigación sobre la broca del café en México: logros, retos y perspectivas. Sociedad Mexicana de Entomología y El Colegio de la Frontera Sur. Chiapas, México, 1-13.

Barrera, J.F., Herrera, J., Villacorta, A., García, H., Cruz, L., Barrera, J., Montoya, P., 2006. Trampas de metanol-etanol para detección, monitoreo y control de la broca del café *Hypothenemus hampei*. Sociedad Mexicana de Entomología y El Colegio de la Frontera Sur Manzanillo, Colima, México, 71-83.

Beer, J., Muschler, D., Kass, D., Somarriba, E., 1998. Shade management in coffee and cacao plantations. Agrofor. Syst. 38, 139-164.

Benavides, P., Bustillo, A., Cárdenas, R., Montoya, E., 2003. Análisis biológico y económico del manejo integrado de la broca del café en Colombia. Cenicafé 54, 5 - 23.

Berndt, L.A., Wratten, S.D., 2005. Effects of alyssum flowers on the longevity, fecundity, and sex ratio of the leafroller parasitoid *Dolichogenidea tasmanica*. Biol. Control 32, 65-69.

Bosselman, A.S., Dons, K., Oberthur, T., Smith, C., Ræbild, A., Usma, H. 2009. The influence of shade trees on coffee quality in small holder coffee agroforestry systems in Southern Colombia. Agric. Ecosyst. Environ. 129, 253-260.

Brun, L., Stuart, J., Gaudichon, V., Aronstein, K., French-Constant, R., 1995. Functional haplodiploidy: a mechanism for the spread of insecticide resistance in an important international insect pest. Proc. Natl. Acad. Sci. 92, 9861-9865.

Burgiel, S.W., Muir, A.A., 2010. Invasive species, climate change and ecosystem-based adaptation: Addressing multiple drivers of global change. Global Invasive Species Programme, Washington, DC, US and Nairobi, Kenya, p. 55.

Bustillo, A., Cárdenas, R., Villalba, D., Benavides, P., Orozco, J., Posada, F., 1998. Manejo integrado de la broca del café *Hypothenemus hampei* (Ferrari) en Colombia. Cenicafé, 134.

Camilo, J.E., Olivares, F.F., Jiménez, H.A., 2003. Fenología y reproducción de la broca del café (*Hypothenemus hampei* Ferrari) durante el desarrollo del fruto. Agron. Mesoam. 14, 59-64.

Charnov, E., Los-den Hartogh, R., Jones, W., Van den Assem, J., 1981. Sex ratio evolution in a variable environment. Nature 289, 27-33.

Constantino, L., Gil, Z., Jaramillo, A., Benavides, P., Bustillo, A., 2010. Efecto del fenómeno El Niño y La Niña en la dinámica de infestación de la Broca del Café

Hypothenemus hampei (Coleoptera: Curculionidae) en un gradiente altitudinal en la cordillera central Colombiana. Sociedad Colombiana de Entomología, Bogotá, Colombia, 124.

Constantino, L., Gil, Z., Jaramillo, A., Benavides, P., Bustillo, A., 2011a. Efecto del cambio y la variabilidad climática en la dinámica de infestación de la broca del café, *Hypothenemus hampei* en la zona cafetera de Colombia. Sociedad Colombiana de Entomología, Manizales, Colombia, 106-121.

Constantino, L.M., Navarro, L., Berrio, A., Acevedo, F.E., Rubio, D., Benavides, P., 2011b. Aspectos biológicos, morfológicos y genéticos de *Hypothenemus obscurus* e *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae). Rev. Colomb. Entomo. 1 37, 173-182.

Cure, J.R., Santos, R.H., Moraes, J.C., Vilela, E.F., Gutierrez, A.P., 1998. Fenologia e dinâmica populacional da broca do café *Hypothenemus hampei* (Ferr.) relacionadas às fases de desenvolvimento do fruto. An. Soc. Entomol. Brasil 27, 325 - 335.

DaMatta, F., Rena, A., Zambolim, L., 2002. Ecofisiologia de cafezais sombreados ea pleno sol. O estado da arte de tecnologias na produção de café. Universidade Federal de Viçosa, Viçosa, 93-135.

DaMatta, F., Rodríguez, N., 2007. Producción sostenible de cafetales en sistemas agroforestales del Neotrópico: una visión agronómica y eco fisiológica. Agron. Colomb. 25, 113-123.

DaMatta, F., Ronchi, C., Maestri, M., Barros, R., 2007. Ecophysiology of coffee growth and production. Braz. J. Plant. Physiol. 19, 485-510.

Damon, A., 2000. A review of the biology and control of the coffee berry borer, *Hypothenemus hampei* (Coleoptera : Scolytidae). B. Entomol. Res. 90, 453-465.

De la Rosa, W., Alatorre, R., Trujillo, J., Barrera, J., 1997. Virulence of *Beauveria bassiana* (Deuteromycetes) strains against the coffee berry borer (Coleoptera: Scolytidae). J. Econ. Entomol. 90, 1534-1538.

Ebbert, M.A., Wensch, D., 1993. Endosymbiotic sex ratio distorters in insects and mites. In: Wensch, D.L., Ebbert, M.A. (Eds.), Evolution and diversity of sex ratio in insects and mites. Chapman Hall, New York, pp. 150-191.

Edgington, S., Segura, H., De la Rosa, W., Williams, T., 2000. Photoprotection of *Beauveria bassiana*: testing simple formulations for control of the coffee berry borer. Int. J. Pest Manage 46, 169-176.

Frazer, G.W., Canham, C., Lertzman, K., 1999. Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York 36.

- Féliz, M.D., Guharay, F., Beer, J. 2004. Incidence of the coffee berry borer (*Hypothenemus hampei*) in coffee plants under different shade types in San Marcos, Nicaragua. *Agroforesteria en las Americas* 41-42, 56-61.
- Geromel, C., Ferreira, L.P., Davrieux, F., Guyot, B., Ribeyre, F., Brígida dos Santos Scholz, M., Protasio Pereira, L.F., Vaast, P., Pot, D., Leroy, T., 2008. Effects of shade on the development and sugar metabolism of coffee (*Coffea arabica* L.) fruits. *Plant Physiol. and Biochem.* 46, 569-579.
- Gingerich, D.P., Borsa, P., Suckling, D.M., Brun, L.-O., 1996. Inbreeding in the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae) estimated from endosulfan resistance phenotype frequencies. *B. Entomol. Res.* 86, 667-674.
- Greenberg, R., Bichier, P., Angon, A.C., Reitsma, R., 1997. Bird populations in shade and sun coffee plantations in central Guatemala. *Conserv. Biol.* 11, 448-459.
- Haraprasad, N., Niranjana, S., Prakash, H., Shetty, H.S., Wahab, S., 2001. *Beauveria bassiana* a potential mycopesticide for the efficient control of coffee berry borer, *Hypothenemus hampei* (Ferrari) in India. *Biocontrol Sci. Tech.* 11, 251-260.
- Harmsen, E.W., Miller, N.L., Schlegel, N.J., Gonzalez, J., 2009. Seasonal climate change impacts on evapotranspiration, precipitation deficit and crop yield in Puerto Rico. *Agric Water Manag.* 96, 1085-1095.
- Hunter, M.D., Price, P.W., 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73, 723-732.
- Inglis, G., Goettel, M., Butt, T., Strasser, H., 2001. Use of hyphomycetous fungi for managing insect pests. In: Butt, T., Jackson, C., and N. Magan (Ed.), *Fungi as biocontrol agents: Progress, Problems and Potential* CABI Publishing, Oxon, UK, pp. 23-69.
- Jaramillo, J., Borgemeister, C., Baker, P., 2006. Coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): searching for sustainable control strategies. *B. Entomol. Res.* 96, 223-233.
- Jaramillo, J., Chabi-Olaye, A., Kamonjo, C., Jaramillo, A., Vega, F.E., Poehling, H.M., Borgemeister, C., 2009. Thermal Tolerance of the Coffee Berry Borer *Hypothenemus hampei*: Predictions of Climate Change Impact on a Tropical Insect Pest. *Plos One* 4, e6487.
- Jaramillo, J., Muchugu, E., Vega, F.E., Davis, A., Borgemeister, C., Chabi-Olaye, A., 2011. Some like it hot: The influence and implications of climatic change on coffee berry borer (*Hypothenemus hampei*) and coffee production in east Africa. *Plos One* 4, 1-11.
- Jaramillo, J., Setamou, M., Muchugu, E., Chabi-Olaye, Jaramillo, A., Makubana, J., Maina, J., Gathara, S., Borgemeiste, C. 2013. Climate change or Urbanization? Impacts

on a traditional coffee production system in East Africa over the Last 80 Years. Plos One 8, e 51815.

Johnson, M., Kellermann, J., Stercho, A., 2010. Pest reduction services by birds in shade and sun coffee in Jamaica. Anim. Conserv. 13, 140-147.

Kageyama, D., Narita, S., Watanabe, M., 2012. Insect sex determination manipulated by their endosymbionts: incidences, mechanisms and implications. Insects 3, 161-199.

King, B., D'Souza, J., 2004. Effects of constrained females on offspring sex ratios of *Nasonia vitripennis* in relation to local mate competition theory. Can. J. Zool. 82, 1969-1974.

Kirkendall, L., Wrensch, D., Ebbert, M., 1993. Ecology and evolution of biased sex ratios in bark and ambrosia beetles. In: Wrensch, D.L., Ebbert, M.A. (Eds.), Evolution and diversity of sex ratio in insects and mites. Chapman Hall, New York, pp. 235 - 345.

Larsen, A., Philpott, S.M., 2010. Twig - Nesting Ants: The Hidden Predators of the Coffee Berry Borer in Chiapas, Mexico. Biotropica 42, 342-347.

Letourneau, D.K., Jedlicka, J.A., Bothwell, S.G., Moreno, C.R., 2009. Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annu Rev. Ecol. Evol. Syst. 40, 573-592.

Mathieu, F., Brun, L.-O., Frerot, B., Suckling, D., Frampton, C., 1999. Progression in field infestation is linked with trapping of coffee berry borer, *Hypothenemus hampei* (Col., Scolytidae). J. Appl. Entomol. 123, 535-540.

Muñoz, R., 1989. Ciclo biológico y reproducción partenogenética de la broca del fruto de cafeto *Hypothenemus hampei* (Ferr.). Turrialba 39, 415 - 421.

NAPPO, 2007. Detection of Coffee Berry Borer, *Hypothenemus hampei*, in Puerto Rico - United States. Official Pest Reports (<http://www.pestalert.org/oprDetail.cfm?oprID=281>).

Nigro, R.G., Campos, M.C.C., Perondini, A.L.P., 2007. Temperature and the progeny sex-ratio in *Sciara ocellaris* (Diptera, Sciaridae). Genet. Mol. Biol. 30, 152-158.

Normark, B.B., 2003. The evolution of alternative genetic systems in insects. Annu. Rev. Entomol. 48, 397-423.

Pardee, G.L., Philpott, S.M., 2011. Cascading indirect effects in a coffee agroecosystem: effects of parasitic phorid flies on ants and the coffee berry borer in a high-shade and low-shade habitat. Environ. Entomol. 40, 581-588.

Perfecto, I., Rice, R.A., Greenberg, R., Van der Voort, M.E., 1996. Shade Coffee: A Disappearing Refuge for Biodiversity. BioScience 46, 598 - 608.

Philpott, S.M., Arendt, W.J., Armbrrecht, I., Bichier, P., Diestch, T.V., Gordon, C., Greenberg, R., Perfecto, I., Reynoso, R., Soto-Pinto, L., 2008. Biodiversity loss in Latin

American coffee landscapes: review of the evidence on ants, birds, and trees. *Conserv. Biol.* 22, 1093-1105.

Pucheta, M., Flores Macías, A., Rodríguez Navarro, S., De La Torre, M., 2006. Mecanismo de acción de los hongos entomopatógenos. *Interciencia* 31, 856-860.

R core Team, 2013. R: A language and environment for statistical computing. R foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0 (<http://www.R.project.org/>).

Rodríguez, D., Cure, J.R., Gutierrez, A.P., Cotes, J.M., Cantor, F., 2013. A coffee agroecosystem model: II. Dynamics of coffee berry borer. *Ecol. Modell.* 248, 203-214.

Rosenfeld, C.S., Roberts, R.M., 2004. Maternal diet and other factors affecting offspring sex ratio: a review. *Biol. Reprod.* 71, 1063-1070.

Ross, L., Dealey, E.J., Beukeboom, L.W., Shuker, D.M., 2011. Temperature, age of mating and starvation determine the role of maternal effects on sex allocation in the mealybug *Planococcus citri*. *Behav. Ecol. Sociobiol.* 65, 909-919.

Ruiz-Cárdenas, R., Baker, P., 2010. Life table of *Hypothenemus hampei* (Ferrari) in relation to coffee berry phenology under Colombian field conditions. *Sci. Agr.* 67, 658-668.

Sanchez, E., Dufour, B., Olivas, A., Virginio-Filho, E. Vilchez, S., Avelino, J. 2013. Shade has antagonistic effects on the coffee berry borer. International Conference on Coffee Science. San Jose, Costa Rica, 729-736.

SERCC, 2007. Historical Climate Summaries for Puerto Rico and the US Virgin Island. University of North Carolina at Chapel Hill. Available for download at https://www.sercc.com/climateinfo/historical/historical_pr.html

Siles, P., Harmand, J., Vaast, P. 2010. Effects of *Inga densiflora* on the microclimate of coffee (*Coffea arabica* L.) and overall biomass under optimal growing conditions in Costa Rica. *Agroforest. Syst.* 78, 269-286.

Silva, N., Frizzas, M., Oliveira, C., 2011. Seasonality in insect abundance in the "Cerrado" of Goiás State, Brazil. *Rev. Bras. Entomol.* 55, 79-87.

Soto-Pinto, L., Perfecto, I., Caballero-Nieto, J., 2002. Shade over coffee: its effects on berry borer, leaf rust and spontaneous herbs in Chiapas, Mexico. *Agroforest Syst.* 55, 37-45.

Sponagel, K., 1994. La broca del café *Hypothenemus hampei* en plantaciones de café robusta en la Amazonía Ecuatoriana. Wissenschaftlicher, Fachverlag, Giessen, Ger, p. 191.

Teodoro, A., Klein, A.-M., Tschardtke, T., 2008. Environmentally mediated coffee pest densities in relation to agroforestry management, using hierarchical partitioning analyses. *Agric. Ecosyst. Environ.* 125, 120-126.

Teodoro, A., Klein, A., Reist, P., Tschardt, T., 2009. Agroforestry management affects coffee pests contingent on season and developmental stage. *Agric. For. Entomol.* 11, 295-300.

Uemura-Lima, D.H., Ventura, M.U., Mikami, A.Y., Da Silva, F.C., Morales, L., 2010. Responses of Coffee berry borer, *Hypothenemus hampei* (Ferrari)(Coleoptera: Scolytidae), to vertical distribution of methanol: ethanol traps. *Neotrop. Entomol.* 39, 930-933.

Vaast, P., Bertrand, B., Perriot, J.J., Guyot, B., Genard, M., 2006. Fruit thinning and shade improve bean characteristics and beverage quality of coffee (*Coffea arabica* L.) under optimal conditions. *J. Sci. Food Agric.* 86, 197-204.

Valdés, B.C., Ravelo, H.G., 2006. Caracterización de la dinámica poblacional de la broca del café (*Hypothenemus hampei* (Ferrari)(Curculionidae: Scolytinae) durante el desarrollo de los frutos. *Centro Agrícola* 33, 55-60.

Vázquez, L.L., Alfonso, J., Ramos, Y., Martínez, A., Moreno, D., Matienzo, Y., 2012. Relaciones de *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae) con el suelo del cafetal como base para su manejo agroecológico. *Agroecología* 7, 81-90.

Vega, F.E., Benavides, P., Stuart, J.A., O'Neill, S.L., 2002. *Wolbachia* infection in the coffee berry borer (Coleoptera: Scolytidae). *Ann. Entomol. Soc. Am.* 95, 374-378.

Vega, F.E., Infante, F., Castillo, A., Jaramillo, J., 2009. The coffee berry borer, *Hypothenemus hampei* (Ferrari)(Coleoptera: Curculionidae): a short review, with recent findings and future research directions. *Terr. Arthropod. Rev.* 2, 129-147.

Vega, F.E., Posada, F., Aime, M.C., Pava-Ripoll, M., Infante, F., Rehner, S.A., 2008. Entomopathogenic fungal endophytes. *Biol. Control* 46, 72-82.

Webge, K., Cilas, C., Decazy, B., Alauzet, C., Dufour, B., 2003. Estimation of Production Losses Caused by the Coffee Berry Borer (Coleoptera: Scolytidae) and Calculation of an Economic Damage Threshold in Togolese Coffee Plots. *J. Econ. Entomol.* 96, 1473 - 1478.

Weeks, A.R., Velten, R., Stouthamer, R., 2003. Incidence of a New Sex-ratio-distorting Endosymbiotic Bacterium among arthropods. *Proc. R. Soc. Lond. [Biol]* 270, 1857-1865.

Werren, J.H., Baldo, L., Clark, M.E., 2008. *Wolbachia*: master manipulators of invertebrate biology. *Nat. Rev. Microbiol.* 6, 741-751.

Wrigley, G., 1988. Coffee. Tropical Agriculture Series. Longman Scientific & Technical and John Wiley & Sons, New York, pp. 483 - 443.

Figures captions

Fig 1. Monthly changes in infestation (a) and CBB per fruit (b) of the coffee berry borer (*Hypothenemus hampei* F.) in shade vs. sun coffee. Data from 2010 and 2011 are combined. Means $\pm$ SE are shown. Asterisks indicate a significant effect of coffee agroecosystem (sun vs. shade) on the abundance of individuals. Generalized linear mixed models: ***= $p < 0.001$, **= $p < 0.01$, * = $p < 0.05$, · = $p < 0.1$

Fig 2. Population structure of the coffee berry borer (*Hypothenemus hampei* F.). Data from 2010 and 2011 are combined. Means + SE are shown. Asterisks indicate a significant effect of coffee agroecosystem (sun vs. shade) on the abundance of individuals. Generalized linear mixed models: ***= $p < 0.001$, **= $p < 0.01$, * = $p < 0.05$, · = $p < 0.1$.

Fig 3. Proportion of coffee berry borer (*Hypothenemus hampei* F.) colonizing females in positions A, B, C and D. (a) sun vs. shade; (b) shade coffee and (c) sun coffee showing changes during the growing season. Data from 2010 and 2011 are combined.

Fig 4. Monthly averages of captured females of *Hypothenemus hampei* in traps in sun vs. shade coffee. Stages of coffee phenology in Puerto Rico are shown above the bars. Data from 2010 and 2011 are combined. Y scale was cut from 100 to 150.

Figure 1

Figure 2

Figure 3

Figure 4

Table 1- Environmental variables measured in the six study plots for the two coffee agroecosystems (sun and shade). Mean $\pm$ S.E. are given for daily temperature range and relative humidity.

Coffee agroecosystem		Temperature (°C)			Humidity (%)		Coffee variety	Coffee plants density ^c
		Min	Max	Daily range ^a	Min	Mean		
Shade	F1	15.7	29.7	7.12 $\pm$ 0.2	56.3	94.7 $\pm$ 0.1	catuai	11
	F2	15.2	30.7	9.94 $\pm$ 0.2	52.3	90.8 $\pm$ 0.2	caturra	10
	F3	15.2	29.9	8.07 $\pm$ 0.2	57.7	92.5 $\pm$ 0.1	caturra	12
Sun	F1	14.7	36.2	13.8 $\pm$ 0.3	38.5	89.4 $\pm$ 0.3	catuai	12
	F2	12.8	38.2	16.8 $\pm$ 0.3	26.7	83.6 $\pm$ 0.3	caturra	10
	F3	13.8	35.4	13.7 $\pm$ 0.3	34.9	88.3 $\pm$ 0.2	caturra	13
Statistics ^b		F=13.6	F=3.47	F=3.40	F=620.2	F=248.7	-	-
		<i>p</i> <0.001	<i>p</i> = 0.03	<i>p</i> =0.03	<i>p</i> <0.001	<i>p</i> <0.001		

^a Daily temperature range calculated as maximum – minimum daily temperatures

^b Statistics based on differences between sun and shade

^c Number of coffee plants in a transect of 20 meters

Table 2. GLMM estimates for coffee berry borer (*Hypothenemus hampei*) infestation and total number of individuals per fruit according to coffee agroecosystem (sun and shade) and sampling dates.

Model variable	CBB infestation			Total population of CBB per fruit		
	Estimate	Z	p	Estimate	Z	p
sun vs. shade	- 1.182	4.86	< 0.001***	0.170	10.16	< 0.001***
Sampling dates	0.284	18.76	< 0.001***	0.077	25.30	< 0.001***

Generalized linear mixed models: ***= $p < 0.001$, **= $p < 0.01$, * = $p < 0.05$, · = $p < 0.1$

Table 3. Mean $\pm$ S.E. of infestation, total population per fruit, sex ratio and percentage of fruits without males of the coffee berry borer *Hypothenemus hampei* according to coffee agroecosystem (sun and shade) and year.

Variable	Shade coffee		Sun coffee	
	2010	2011	2010	2011
Mean $\pm$ s.e. of % infestation per plot	7.20 $\pm$ 0.42	51.95 $\pm$ 1.53	4.51 $\pm$ 0.33	26.03 $\pm$ 1.11
Mean $\pm$ s.e. of total # CBB per fruit	5.13 $\pm$ 0.32	5.33 $\pm$ 0.32	7.27 $\pm$ 0.32	6.32 $\pm$ 0.36
Mean $\pm$ s.e. of Sex ratio ^a	0.79 $\pm$ 0.02	0.75 $\pm$ 0.03	0.86 $\pm$ 0.01	0.81 $\pm$ 0.03
Mean $\pm$ s.e. of % fruits without males ^b	65.6 $\pm$ 11.3	77.5 $\pm$ 6.11	61.6 $\pm$ 7.94	80.7 $\pm$ 4.98

^a Calculated as the proportion of females in fruits with progeny and only includes those with newly emerged and adults. We did not include the fruits in which we observed only the colonizing females and females with immature progeny (eggs, larvae and pupae) in which sex cannot be determined visually.

^b (# fruits without males/ # fruits with progeny of juveniles and adults) x100

Table 4. Mean $\pm$ S.E. of total population per fruit of the coffee berry borer (*Hypothenemus hampei*) according to coffee agroecosystem (sun and shade) and fruit color or maturity stage.

Type of coffee	Total population of CBB per fruit		
	Green	Yellow	Red
Shade coffee	4.86 $\pm$ 0.49	6.61 $\pm$ 0.93	7.63 $\pm$ 0.74
Sun coffee	5.51 $\pm$ 0.62	7.53 $\pm$ 0.91	8.37 $\pm$ 0.75

Appendices

Table A.1- Species and family of trees associated with shade plots.

Farm	Species and Family	Family
F1	<i>Guarea guidonia</i>	Meliaceae
	<i>Cyathea arborea</i>	Cyatheaceae
	<i>Inga vera</i>	Fabaceae
	<i>Thespesia grandiflora</i>	Malvaceae
	<i>Spathodea campanulata</i>	Bignoniaceae
	<i>Miconia prasina</i>	Melastomataceae
	<i>Cordia sulcata</i>	Boraginaceae
	<i>Cecropia schreberiana</i>	Urticaceae
	<i>Musa x paradisiaca</i>	Musaceae
F2	<i>Tabebuia heterophylla</i>	Bignoniaceae
	<i>Inga vera</i>	Fabaceae
	<i>Inga laurina</i>	Fabaceae
	<i>Roystonea borinquena</i>	Arecaceae
	<i>Spathodea campanulata</i>	Bignoniaceae
	<i>Artocarpus altilis</i>	Moraceae
	<i>Musa x paradisiaca</i>	Musaceae
	<i>Citrus paradisi</i>	Rutaceae
F3	<i>Inga vera</i>	Fabaceae
	<i>Inga laurina</i>	Fabaceae
	<i>Tabebuia heterophylla</i>	Bignoniaceae
	<i>Artocarpus altilis</i>	Moraceae
	<i>Citrus sinensis</i>	Rutaceae
	<i>Spathodea campanulata</i>	Bignoniaceae
	<i>Tectona grandis</i>	Lamiaceae
	<i>Andira inermis</i>	Fabaceae