The Coffee Berry Borer (Coleoptera: Curculionidae) in Puerto Rico: Distribution, Infestation, and Population per Fruit

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Received 25 October 2016; Editorial decision 11 December 2016

Abstract

The coffee berry borer (CBB) (Hypothenemus hampei: Ferrar) was first detected in Puerto Rico in 2007. Its distribution since then has been extensive, but not extensively documented. An island-wide survey was carried out from August to November 2014 (the coffee production season) to assess CBB distribution, infestation, and population per fruit. The CBB was well-established throughout the coffee-growing area of Puerto Rico, but was not evenly distributed. Infestation (or percentages of fruits perforated) in sites sampled ranged from 0 to 95%, and CBB number per infested fruit varied from 1 to 34 individuals. CBB infestation and total population per fruit were positively correlated with altitude. Highest infestation and total population were observed in sites located >400 masl; most of the coffee-producing area in Puerto Rico is above this altitude. Coffea arabica (L.) had higher CBB infestation and population per fruit than Coffea canephora (Pierre ex A. Froehner) (robusta coffee). Based on these results, management tools should be implemented to mitigate the severe damage that CBB is causing in Puerto Rico. These management tools should include the removal of all fruits that remain on the plants after harvest and the use of the entomopathogenic fungus Beauveria bassiana (Ball.) Vuill. for biocontrol, especially on coffee farms at higher elevations.

Key words: Beauveria bassiana, biocontrol, Coffea arabica, Coffea canephora, Hypothenemus hampei

Coffee was introduced to Puerto Rico in 1736 and the coffee industry was initiated in the early 1880s (Bergad 1978, Dietz, 1986). By the end of the 19th century, coffee was the chief commercial crop, and Puerto Rican coffee was among the most highly prized in the world (Dietz 1986). Today coffee is the fifth most valuable crop in Puerto Rico, and is a pillar of the economy of the central mountain region (Flores 2011).

In the last few decades, coffee production in Puerto Rico has suffered setbacks due to socioeconomic problems such as lack of harvesters and increased costs of production. Diseases, mainly the coffee leaf rust (Hemileia vastatrix) (Berk. & Broome), and insect pests, especially the coffee leaf miner (Leucoptera coffeella) (Guérin-Méneville), have also affected production (Gallardo 1988, Rodriguez & Monroig 1991, Gallardo et al. 2008, James et al. 2015). The arrival of the coffee berry borer in 2007 was another blow to coffee production on the Island. The coffee industry in Puerto Rico is at risk: from 2007 to 2012, 1,200 coffee farms were abandoned and the total value of Puerto Rican coffee fell by $12.6 million, or 33% (USDA 2014).

The coffee berry borer (CBB) Hypothenemus hampei (Ferrar) (Coleoptera: Curculionidae: Scolytinae), is endemic to Africa, as is coffee (Le Pelley 1968, Damon 2000, Vega et al. 2009). This insect was first reported as a coffee pest in 1901 in the Republic of Congo (Fleutiaux 1901, Le Pelley 1968). CBB was first detected in the Americas in Brazil in 1913 (Infante et al. 2014) and now is present in all coffee-growing countries except China and Nepal (Jaramillo et al. 2011), with the most recent colonization in Hawaii in 2010 (Burbano et al. 2011). In Puerto Rico, the CBB was first detected in the municipalities of San Sebastian and Utuado in 2007 (NAPPO 2007). Seven years later, the extent to which the CBB has colonized the coffee-growing area of the Island has still not been fully documented, and little has been published about the extent of damage to the coffee crop.

CBB distribution and crop damage are related to several environmental factors. For example, there were positive relationships between CBB infestation, altitude, and population per fruit. The CBB was well-established throughout the coffee-growing area of Puerto Rico, but was not evenly distributed. Infestation (or percentages of fruits perforated) in sites sampled ranged from 0 to 95%, and CBB number per infested fruit varied from 1 to 34 individuals. CBB infestation and total population per fruit were positively correlated with altitude. Highest infestation and total population were observed in sites located >400 masl; most of the coffee-producing area in Puerto Rico is above this altitude. Coffea arabica (L.) had higher CBB infestation and population per fruit than Coffea canephora (Pierre ex A. Froehner) (robusta coffee). Based on these results, management tools should be implemented to mitigate the severe damage that CBB is causing in Puerto Rico. These management tools should include the removal of all fruits that remain on the plants after harvest and the use of the entomopathogenic fungus Beauveria bassiana (Ball.) Vuill. for biocontrol, especially on coffee farms at higher elevations.
between CBB infestation and altitude (Damon 2000, Constantin et al. 2011, Jaramillo et al. 2011), positive relationships between the number of CBB individuals and temperature (Teodoro et al. 2008, 2009; Jaramillo et al. 2011), and higher infestation levels in dry years compared with wetter years (Constantino 2010, Constantin et al. 2011, Rodríguez et al. 2013, Marín et al. 2016). These studies also show substantial differences among countries in CBB damage, though sampling methods vary among studies.

Thus, the objectives of this study were to determine the distribution, infestation (percentage of fruits perforated by CBB) and population per fruit (total number of individuals in all developmental stages) in Puerto Rico. We asked the following questions:

1. How is the CBB distributed in Puerto Rico? Seven years after the first report of the CBB in Puerto Rico, we predicted that the CBB would be present in all coffee-growing areas of the Island.

2. Is altitude correlated with the infestation and total population per fruit of CBB? We hypothesized that CBB infestation and population per fruit would increase with altitude, due to more favorable temperatures at higher altitudes.

3. Do infestation and total CBB population per fruit differ between Coffea arabica (L.) and Coffea canephora (Pierre ex A. Froehner) (robusta coffee)? Results from previous studies are contradictory, so we predicted differences would not be significant.

4. Does CBB population per fruit differ between fruits remaining on coffee plants and fallen fruits on the ground? We hypothesized that the population would be higher in fruits remaining on the plants, due to decomposition of fallen fruits on the ground.

Materials and Methods

Study Area

The main coffee-growing area in Puerto Rico is the central-west region of the Cordillera Central (or Central Mountain Range) (Muniz & Monroig 1994, Flores 2011) (Fig. 1). A survey of CBB was carried out in this area from August to November 2014. We sampled 214 sites in 97 farms and rural areas in 17 municipalities (one sampling per site) (Fig. 1). The sites sampled were predominantly C. arabica. The most common cultivars were Limani, Catuра, Bourbon (or Bourbon), and Catuaí. The cultivars Puerto Rico, Frontón, Pacas and Típica were less common. Twenty-six of the 214 sites were C. canephora (robusta coffee).

Sites ranged from 55 to 966 m above sea level (masl) (Table 1), including the lowest and some of the highest coffee farms in Puerto Rico. Both shade and sun coffee were sampled; the effect of shade was discussed previously (Marín et al. 2016). Growers were asked about CBB management, especially the use of the fungus Beauveria bassiana (Bals.) Vuill. In Puerto Rico, B. bassiana is applied as the commercial formulation Mycotrol.

Climatic records of annual minimum, maximum, and mean temperatures for each municipality were acquired from xMACIS (http://xmacis.rcc-acis.org/, managed by the NOAA Regional Climatic Centers) (Table 1).

Infestation

For sampling at sites planted with C. arabica, we delimited a plot 20 × 20 m. In the case of C. canephora, it was difficult to delimit plots, since the majority of plants sampled were along roads and on the borders of C. arabica sites. For both species, in each site, we collected one branch at breast height from three randomly selected plants. The total number of fruits and CBB-bored fruits on each branch were counted. Infestation was defined as the percent of fruits perforated by CBB; this definition is consistent with the existing literature (Vega et al. 2009, Larsen & Philpott 2010, Jaramillo et al. 2013, Aristizábal et al. 2016).

Population per Fruit

We sampled 20 bored fruits from the three branches collected from each site (or all bored fruits when there were <20). A total of 2,738 fruits were dissected with aid of a dissecting microscope at 30–50×. CBB population per fruit was defined as the total number of individuals in all developmental stages (eggs, larvae, pupae, and adults) in a single fruit. This definition is consistent with the existing literature (Sanchez et al. 2013, Marín et al. 2016).

In addition, we counted CBB in coffee fruits of C. arabica remaining on the plants and fallen fruits on the ground after harvest, which have been reported to be reservoirs for CBB between coffee crops. These samples were collected in one farm each in four municipalities: Adjuntas (18◦10′42.5″N, −66◦44′36.3″W), Ciales (18◦18′15.84″N, −66◦32′40.32″W), Utuado (18◦15′54.63″N, −66◦47′20.24″W), and Yauco (18◦8′55.79″N, −66◦50′32.75″W). Samples were collected monthly from January to April 2015 (the period between coffee crops), 20 fruits from the branches and 20 fallen fruits, and CBB individuals at each stage of development were counted.

Data Analysis

For statistical analysis, we considered CBB infestation and population as response variables. The explanatory variables were altitude, coffee species (C. arabica vs C. canephora), and application of B. bassiana (Mycotrol) within the last year, as reported by growers.

To analyze the effect of coffee species (C. arabica vs C. canephora) on CBB infestation and population per fruit, we used generalized linear mixed models (GLMM) with a binomial and Poisson errors distribution, respectively. We compared sites located at the same altitude and municipality. The variable coffee species (C. arabica vs C. canephora) was included as a fixed factor and altitude as a random factor.

The effect of the application of Mycotrol on CBB infestation and population was measured using GLMM with binomial and Poisson errors distributions, respectively. As with comparisons between coffee species, for each site to which Mycotrol had been applied at least once, we selected a control site without Mycotrol in the same municipality and altitude; altitude was included in the model as a random factor.

Mycotrol use was only documented in sites planted with C. arabica.

To compare the population of CBB between unharvested and fallen fruits, we used a generalized linear model (GLM) with a Poisson errors distribution. Relationships of altitude vs CBB infestation and population per fruit were tested using Spearman’s correlation analysis.

Data from C. arabica and C. canephora were analyzed separately. All analyses were performed in R (R core Team 2013).

Results

Distribution and Infestation of the Coffee Berry Borer

CBB was present in all sites sampled throughout the main coffee-growing region of Puerto Rico. However, damage was not uniformly distributed. Considering data for both coffee species together, we observed that infestation ranged from 0 to 95% and infestation in almost 40% of sites was greater than 20% (Fig. 2a).
Correlation analysis showed a significant and positive relationship between altitude and CBB infestation for both coffee species: *C. arabica* ($r = 0.35; P < 0.001$) and *C. canephora* ($r = 0.60; P = 0.001$). For *C. arabica*, the infestation was higher than 20% in sites located above 400 masl (Fig. 3a); most coffee production in Puerto Rico occurs above this altitude (Table 1).

The mean infestation of CBB by municipality is shown in Table 2. Highest average infestation per municipality was observed in (in descending order) Yauco (30.5%), Utuado, Maricao, Jayuya, and Adjuntas (24.3%). Most sites surveyed in these municipalities (62%) were >400 masl. The CBB was not present in the sites sampled in Barceloneta, Rio Grande, and Vega Baja, which were <200 masl. These municipalities are not considered part of the normal area of coffee production (Muñiz & Monroig 1994).

**Population per Bored Fruit**

More than one adult and/or immature stages of the CBB were observed in 62% of perforated fruits. In 66% of sites sampled, the maximum number of individuals per fruit was >10 (Fig. 2b). The highest mean and maximum CBB population per fruit was observed in the municipalities where infestation was the highest; for example, in Yauco, Ciales, Jayuya, and Utuado, the largest number of CBB observed in a single fruit was >30 (Table 2).

Similar to infestation, there was a significant and positive relationship between altitude and CBB population per fruit. This was true for both coffee species, *C. arabica* ($r = 0.39; P < 0.001$) and *C. canephora* ($r = 0.67; P = 0.001$). We found almost the double the number of individuals in sites above 201 masl than at sites below 200 masl (Fig. 3b). Thus, at higher elevations, there is higher CBB infestation and these fruits contain larger number of individuals.

**CBB Populations in Unharvested and Fallen Fruits**

The estimated population of individuals in fruits remaining on plants after harvest was significantly higher than in fruits collected from the ground (GLM, Poisson errors, $Z = 8.47, P < 0.001$). On
average, 16.0 CBB were found in fruits remaining on plants after harvest and 9.3 in fallen fruits. The most heavily populated dry fruit remaining on a plant contained 94 CBBs, of which 45 were adult females. The most heavily populated fallen fruit contained 52 CBB, including 25 adult females.

Differences Between Arabica and Robusta Coffee in CBB Infestation and Population per Fruit

The estimated probabilities of infestation and CBB population per fruit were significantly higher in arabica than robusta coffee (infestation: GLMM, binomial errors, $Z = -3.69$, $P < 0.001$; CBB population per fruit: GLMM, Poisson errors, $Z = -12.22$, $P < 0.001$). However, up to 47 CBBs were found in a single robusta fruit. The mean infestation and the mean population according to altitude for robusta and arabica are shown in Fig. 3. As expected, the maximum altitude at which robusta was found was lower than that for arabica, at 600 m.

Effect of the Application of Mycotrol on CBB Infestation and Population per Fruit

Mycotrol was applied at least once in 42 sites (19.6%). CBB infestation and population per fruit in sites where Mycotrol was applied were slightly but significantly lower than in similar sites in which Mycotrol was not applied (infestation: GLMM, binomial errors, $Z = -0.40$, $P < 0.001$; CBB population per fruit: GLMM, Poisson errors, $Z = -0.16$, $P < 0.001$). Average CBB infestation percentages in sites with Mycotrol were $17.9 \pm 2.74$ and sites without application $21.1 \pm 2.83$, while the CBB population per fruit was $4.0 \pm 0.2$ and $4.6 \pm 0.3$ individuals.

Discussion

Coffee Berry Borer Distribution in Puerto Rico in 2014

In 2014, the CBB was present in all sites sampled throughout the coffee-growing area of Puerto Rico; however, it was not evenly distributed. Our results showed positive correlations of CBB infestation...
and population with altitude. In sites located less than 200 masl, the CBB infestation was lower than 10% and the mean number of individuals per fruit was < 2; while in sites located over 400 masl, CBB infestation was > 20% and, in some cases, reached 95%, and the mean number of individuals per fruit was 4–6 with a maximum of 35 individuals per fruit (Fig. 3a and b and Table 2). These data suggest that at higher elevations in Puerto Rico, the CBB is a serious problem: there are more fruits bored and these fruits contain more individuals. Most of the coffee-producing area in Puerto Rico is over 400 masl (Table 1).

Previous studies also reported a relationship between CBB infestation and altitude (Damon 2000, Soto-Pinto et al. 2002, Constantino 2010, Jaramillo et al. 2011). In Ethiopia and Colombia, CBB infestation was higher at altitudes below 1,000–1,200 masl and lower above 1,600–1,900 masl (Abebe 1998, Constantino 2010). In Mexico, the CBB was sampled in an altitudinal range similar to that of Puerto Rico (100–1,250 masl), and CBB infestation was higher from 500 to 1,000 masl (Baker et al. 1989). Much less has been reported on the relationship between CBB population per fruit and altitude. We observed a tendency similar to infestation, with almost double the number of individuals in fruits collected at altitudes over 200 masl (Fig. 2b).

The correlation among CBB infestation, population per fruit, and altitude is largely a factor of temperature. The optimal range for CBB survival is between 15 and 32°C, with fastest development from 27 to 30°C (Jaramillo et al. 2009). The mean annual temperature in the coffee-growing areas in Colombia was 21–23°C in sites located under 1,200 masl and <19°C over 1,600 masl (Constantino 2010). In Mexico, a mean annual temperature of 23–25°C was reported in sites located from 500 to 1,000 masl (Baker et al. 1989). Here, in Puerto Rico, the mean annual temperature in sites over 400 masl was 21.4–24°C, with a maximum of 30–32°C, which is suitable and optimal for CBB survival and development. Sites located below 200 m had maximum temperatures over 34°C, which may prejudice CBB survival (Table 1).

Unexpectedly High Infestation in Puerto Rico

The percentage of fruits infested by CBB in Puerto Rico in 2014 ranged from 0% to 95%; in almost 40% of the sites sampled infestation was >20% (Fig. 2a and Table 2). This infestation is higher than previous reports from other countries: for example, in East Africa in 2009–2011, infestation was <1–15% (Jaramillo et al. 2013); in Brazil in 1992–1993, it was 21–32% (Cure et al. 1998). In Colombia, in 1995–1996, it was <2–25% (Benavides et al. 2003), although in southern Colombia in 2006, it was <1% (Bosselmans et al. 2009). Infestation in Costa Rica was 2–10% (Sanchez et al. 2013); and in Guatemala, it was <2–25% (Feliz Matos et al. 2004). In Mexico, in 1993, infestation was 10–15% (Barrera 2005), in 1997, it was 0.1–19% (Soto-Pinto et al. 2002) and, in 2008, it was 5–35% (Larsen & Philpott 2010). Although these studies varied in extent, geographic area, and year, it is remarkable that none of them found levels of CBB infestation as high as reported here for Puerto Rico in 2014.

There seem to be three main reasons for the higher infestation observed in Puerto Rico, apart from the environmental factors discussed above. First, most coffee farms in Puerto Rico used few or no integrated pest management measures in 2014. As part of our survey, growers were asked about CBB management. None applied chemical control, and for almost 80% of the sites, growers said they used no type of control at all. In 42 sites (20%), the entomopathogenic fungus B. bassiana was applied at least once a year (as Mycotrol). Traps with alcohol attractants were used in only 15 sites surveyed (7%).

The second reason for the high infestation of CBB in Puerto Rico is the scarcity of labor for harvesting (Flores 2011), which does not allow a rigorous collection of all remaining fruits after the main harvest. CBB can survive in unharvested fruits on the plants and in fallen fruits on the ground, as we show here; these fruits are important reservoirs of CBB between crops (Damon 2000, Wegbe et al. 2003, Armbricht & Gallego 2007, Larsen & Philpott 2010, Benavides et al. 2012). Most Latin American coffee-producing countries use a method called ‘re-re’ (short for ‘recolección y repase,’ or ‘collect and repeat’) in which all fruits remaining on the plants and on the ground are removed immediately after harvest (Benavides et al. 2003, Jaramillo et al. 2006, Benavides et al. 2012). Implementation of measures for cultural control, including ‘re-re,’ may contribute 80% of success in management of the CBB (Busstill 2006).
Since ‘re–re’ is not implemented in Puerto Rico because of scarcity and cost of labor, these unharvested and fallen fruits are important reservoirs for CBB between coffee crops. Dried fruits remaining on the plants appear to be more important reservoirs of CBB than fallen fruits on the ground, harboring up to 94 individuals compared with up to 52 individuals in fallen fruits collected on the ground. Moreover, we observed that fallen fruits decomposed more quickly than dry fruits on plants. By March, the endosperm of fallen fruits was in an advanced state of decomposition, reducing its ability to support the CBB until the new crop of fruits can be attacked in June, as stated by Gallardo and González (2015). Therefore, unharvested fruits on plants should be given priority; they are also easier to collect than fallen fruits.

The third reason is the relatively recent introduction of the CBB (NAPPO 2007). Natural enemies have not yet established an efficient suppression of the pest, partly because the geographic isolation of Puerto Rico slows the natural migration of new natural enemies (Letourneau 1998).

The use of the fungus B. bassiana in Puerto Rico for CBB biocontrol was not common; the fungus was applied in 20% of farms and usually only once a year. However, our analyses showed that the application of the fungus has a negative effect on CBB infestation and population per bored fruit. The fungus was applied as Mycotrol, which contains a strain of B. bassiana sensitive to high temperatures and most efficient from 21.1 to 26.6°C (Kuepfer 2003); it also requires environments with high relative humidity levels (ORG 2010). These preliminary results suggest that Mycotrol application may be effective in reducing CBB. However, it is possible that the growers who applied Mycotrol were also more diligent in other aspects of cultivation; this result shows an association and not causality.

The fungus B. bassiana in Puerto Rico is applied as Mycotrol. However, local strains of B. bassiana also attack CBB (Gallardo et al. 2010). Our results suggested that the application of Mycotrol is related with reduction in CBB infestation and total population per fruit. Female CBBs are in susceptible positions (positions A and B) during the months of May to July, so these months are the best time to apply the fungus (Mariño et al. 2016).

**Comparison Between Arabica Vs Robusta in CBB Damage**

The two most important species for coffee production worldwide are C. arabica and C. canephora (robusta) (Briandet et al. 1996, Ky et al. 2001, Jaramillo et al. 2011). Relatively few studies have compared CBB damage between these two species, and the published reports are contradictory. For example, Chevalier (1947) (cited by Le Pelley 1973), Baker et al. (1989) and Damon (2000) suggested CBB damage is greater in C. canephora than in C. arabica; Le Pelley (1968) reported the contrary.

We found that both infestation and CBB population per fruit were significantly higher in C. arabica than in C. canephora. In Puerto Rico, there are few extensive plantings of C. canephora; however, it is often found along roads and on the borders of arabica sites. Robusta coffee may be an additional reservoir of CBB between arabica crops, due to its capacity to flower and fruit continuously throughout the year (Baker et al. 1989, Damon 2000).

However, our comparison of arabica and robusta coffee is limited in several ways. First, we surveyed only 26 robusta sites, and some were naturalized plants growing along roads. Second, the robusta plants surveyed in Barceloneta were from a new farm only three years old and far from the nearest coffee farm. It is possible that the CBB will become established there with time, although its low altitude (~120 m) suggests that CBB damage there will not be severe.

**Conclusions and Recommendations**

The CBB infestation in Puerto Rico in 2014 was more severe than previously reported in other coffee-growing countries, from 0 to 95% depending on site and altitude. Its wide distribution is remarkable given that the CBB was first reported in Puerto Rico in 2007. The CBB is causing severe damage to Puerto Rico’s coffee crop, especially at intermediate to high elevations. The CBB is a serious threat to the survival of the coffee industry in the Island.

CBB management in these areas (>400 masl) should focus on (1) sampling and monitoring. The best time to start sampling is 60 days after flowering, usually May or June (Gallardo & González 2015). Artisanal traps with ethanol: methanol (1:1) are also recommended to monitor females flying in search of new fruits to attack (Mariño et al. 2016); 15 traps are recommended for each 0.4 hectares (Gallardo & González 2015). (2) The total removal of fruits remaining on the plants after the main harvest, known as ‘re–re’ (described above) (Gallardo & González 2015). (3) The application of the entomopathogenic fungus B. bassiana. The best time to apply the fungus is May–July, when colonizing females are in positions A and B and are thus more susceptible (Mariño et al. 2016, Gallardo & González 2015). (4) Reducing escape of adult females from harvest and post-harvest coffee-processing facilities. These measures are recommended as part of the integrated management programs (IMP) for the CBB (Benavides & Árevalo 2002, Bustillo 2006, Jaramillo et al. 2006, Aristizábal et al. 2016, Gallardo & González 2015) and have been successfully implemented in other countries (Bergamin 1944, Bustillo et al. 1991, Barrera 1995, Guharay et al. 1996, Barrera et al. 2004, Dufour & Frérot 2008, Aristizábal et al. 2011). However, future research should be focused on determining the efficiency of each measure in order to establish the most cost-efficient CBB management.

The tremendous variation among sites in CBB infestation and population per fruit means that studies restricted to one area may not be representative of others. Similarly, since CBB infestation and population may vary greatly from year to year, depending on precipitation and other factors (Mariño et al. 2016), the results presented here for 2014 may not be characteristic of other years. This variability complicates the understanding of the pest and its interaction with multiple environmental variables, which will require further analysis in order to better predict the dynamics of CBB populations and improve control tactics.

**Acknowledgments**

We are grateful to the growers for their hospitality and permission to sample on their farms, and to Mrs. Marian Martínez and the agronomists of the Agricultural Extension Service in Ciales, Moca, Jayuya, Orocovis, San Sebastián, Villalba, and Yauco for help in contacting the coffee growers. We thank Lesly Colón, Tania Hernández, Amanda Díaz, and Diane Mankin for their participation in field and laboratory work. We are grateful to Stephen A. Rehner (USDA-ARS, Beltsville, MD) and Fernando Gallardo (UPR) for advice and support. This project was supported by USDA-ARS Specific Cooperative Agreements 59-1245-4-082 and 59-1245-4-083 and by a grant from the Puerto Rico Science and Technology Research Trust (PRSTRT).
