A Beetle in a Haystack: Are There Alternate Hosts of the Coffee Berry Borer (*Hypothenemus hampei*) in Puerto Rico?

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Abstract: Can the coffee berry borer (*Hypothenemus hampei*, or CBB) use host plants other than coffee for food and shelter? The use of fruits other than coffee has been reported. However, the validity of these reports depends on accurate identification of CBB, which is sometimes uncertain. In this study we sampled potential alternate hosts in coffee farms in Puerto Rico. Fruits with perforations were collected and examined for the presence of scolytid beetles (Coleoptera: Curculionidae: Scolytinae). Scolytids were identified by morphology and DNA barcoding of the COI gene. Association between the presence of *Inga vera* and *Guarea guidonia* trees and infestation rate of CBB in coffee fruits was evaluated. Food preference tests were performed in the laboratory. A total of 3563 beetles were found and 587 were identified as *Hypothenemus* spp.; of these, 85 identifications were confirmed by DNA barcoding. Twenty-seven of the beetles identified were *H. hampei*, mostly in *I. vera* fruits in periods between coffee crops. Most scolytids identified were *H. obscurus*. In preference tests, some CBB initially penetrated *G. guidonia* fruits, but eventually chose coffee. There was no evidence of feeding or reproduction in fruits of *G. guidonia* or *Cajanus cajan*. The results show that in Puerto Rico it is rare to find CBB in fruits of alternate hosts. The scarcity of coffee fruits in the off-season might cause some CBBs to take refuge in other fruits, but they did not feed or reproduce in them in laboratory tests. Understanding the refugia of CBB in the off-season may be useful for designing effective management strategies.

Keywords: barcoding; *Coffea arabica*; pest management; feeding preferences; monophagy; polyphagy; Scolytidae

1. Introduction

How does the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) survive between crops when there are few or no coffee fruits? This question is key for management of the coffee berry borer (or 'CBB') because this period is a population bottleneck; eliminating refugia would greatly reduce the population available to attack the next crop. This question is still unresolved and underlines the importance of identifying the potential alternative hosts for this species. The CBB is the most serious pest of coffee worldwide, so understanding its refugia is of great practical importance to coffee growers and the coffee industry.

Fruits of *Coffea arabica* (the main commercial species) are produced all year round in places with evenly distributed or biomodal rainfall [1,2], but in areas with a single dry season there is one crop per year [3], which leads to long periods without fruits. In Puerto Rico there is a single crop per year; fruits appear in late May and are present until the harvest, from October to December in most areas [4]. As a result, fruits are scarce for approximately 150 days (January to late May). Furthermore, *H. hampei* waits to penetrate fruits until they reach 20% dry weight content, which takes additional 60 days [5].

These long periods without fruits can be a problem for *H. hampei* survival because they exceed the lifespan of the insect. Longevity of female CBBs is very variable; reports range from 26–70 days [6] to 129 days [7]. Females of *H. hampei* can live 81 days without food [8]. Therefore, *H. hampei* survival in Puerto Rico is difficult to understand because females are without food for at least 210 days.

It has been reported that female CBBs can spend the intercrop season on coffee fruits that remain in the plants, and also in fallen fruits on the soil [9]. However, in Puerto Rico fallen fruits on the soil decompose rapidly due to humidity and do not persist long enough to shelter CBBs until the next crop can be infested [4,10]. In contrast, fruits remaining on plants are an important reservoir and can contain >90 CBBs in a single fruit [10].

The presence of *H. hampei* in plants other than coffee has been reported since the beginning of the 20th century. For instance, there are reports that *H. hampei* can be found in *Phaseolus vulgaris* L., *Lens culinaris* Medik., *Pisum sativum* L., *Arachis hypogaea* L., *Zea mays* L., *Ricinus communis* L., *Abelmoscus esculentus* Moench, *Cola acuminata* (P.Beauv.) Schott & Endl., *Myristica fragrans* Houtt., *Leucaena glauca* Benth., *Gossypium* sp., *Tephrosia* sp., *Crotalaria* sp., *Centrosema* sp., *Dialium englerianum* Henriq., and in some nuts and palm seeds [11–13] (cited by Alonzo [14]) and Ghesquière [15] (cited by Vega et al. [16]).

Alternate host observations are only as valid as the identification of the species in question. Species of scolytid beetles are difficult to identify, and there are 71 species of the subfamily Scolytinae that closely resemble *H. hampei* [11,17] (cited by Alonzo [14]). It is therefore not clear that all previous studies that report *H. hampei* on alternate hosts were looking exclusively at *H. hampei*. Schedl was a well-known entomologist specializing in bark beetles [16], so we can assume that his identifications are reliable. He reported *H. hampei* in 20 genera of plants from the forests of the Democratic Republic of Congo [18]. However, a recent, extensive study of herbarium specimens of potential alternate hosts from tropical Africa found no evidence of CBB in fruits other than *Coffea* species [19].

This study asks whether the CBB can use fruits of plants other than coffee as alternate hosts during the period in which coffee fruits are scarce in Puerto Rico, and more generally, whether plants other than coffee can serve as alternate hosts for *H. hampei*. To test this, we looked for scolytid beetles in fruits of plants commonly used as shade trees in coffee farms (e.g., *Inga vera* Willd., Fabaceae), remnant plants of secondary forests in the farms (e.g., *Guarea guidonia* (L.) Sleumer, Meliaceae), and alternative crops commonly planted along coffee (e.g., *Cajanus cajan* (L.) Huth, Fabaceae); scolytids were identified by morphology and DNA barcoding. It also asks whether CBBs preferred coffee fruits over common alternatives; to test this, preference and feeding tests were performed in the lab. Finally, it asks whether there is a relation between CBB infestation and the presence of common trees on coffee farms, using association tests. Confirmation of the existence of alternate hosts could open the possibility of new forms of cultural control of the pest, through the management of those plants.

2. Materials and Methods

2.1. Plants Evaluated in the Field

Monthly samples were taken from January to December 2015 to evaluate the presence of CBB in alternate hosts in four farms located in different municipalities of Puerto Rico. All farms were commercial coffee farms planted with *Coffea arabica* cvs. Limaní, Catuaí, Frontón, Borbón, and Caturra (the most common cultivars in Puerto Rico). The management of the four farms was similar: glyphosate was applied 3–4 times per year to control weeds, except the farm in Utuado, where weeds were removed manually by machete. Two farms in Ciales and Utuado applied copper to manage the coffee leaf rust

(*Hemileia vastatrix*). None of the growers applied chemicals to control the CBB; the grower in Utuado did not use anything, and the other three growers occasionally used Mycotrol®(active ingredient: The fungus *Beauveria bassiana*) and artisanal traps to reduce CBB populations [4,10] (Table 1).

Table 1. Characteristics of coffee farms sampled. Weather data from xmACIS (http://xmacis.rcc-acis.org/).

Municipality	Coordinates	Altitude (m.a.s.l)	Size (ha.)	Farm Management	Coffee Cultivars	Average Annual Precipitation (mm)	Average Temperature (°C)
Adjuntas	18°10′28.73″ N, 66°44′35.83″ W	510	29	Glyphosate [1] Mycotrol® [2]	Catuaí, Limaní	1930	21
Ciales	18°18′15.18″ N, 66°32′42.26″ W	310	23	Glyphosate Mycotrol®, traps [2] Copper [3]	Catuaí, Limaní	1811	25
Utuado	18°15′51.52″ N, 66°47′14.61″ W	580	6	Mycotrol® Copper	Borbón, Frontón, Limaní	2286	23
Yauco	18°8′57.15″ N, 66°50′35.54″ W	790	10	Glyphosate® Traps	Borbón, Catuaí, Caturra, Limaní	2192	22

[1] Used to control weeds, [2] Used to monitoring and control the coffee berry borer (CBB), [3] Used to control coffee leaf rust (*Hemileia vastatrix*).

Around and within these coffee plots, all plant species with fruits >1 cm in diameter were sampled, except grasses and herbs. Fruits were collected with a pole trimmer up to a height of 5 m. The irregular topography of the farms and distribution of trees precluded a precisely defined distance for sampling. Botanical samples were pressed and taken to the Herbarium of the University of Puerto Rico, Río Piedras (UPRRP) for identification. Up to 50 fruits per species with at least one perforation similar in size to those made by the CBB [19] were collected monthly to evaluate the presence of *H. hampei* in the fruits. The plant species sampled in each farm are listed in Table 2.

Table 2. Plant species with perforated fruits evaluated as potential alternate hosts, and their distribution on the coffee farms sampled.

Plant species	Family	Adjuntas	Ciales	Utuado	Yauco
Cajanus cajan	Fabaceae		X	X	X
Cupania americana	Sapindaceae	X			
Cordia sulcata	Boraginaceae				X
Guarea guidonia	Meliaceae	X	X		X
Inga vera	Fabaceae	X	X	X	X
Miconia serrulata	Melastomataceae		X	X	
Nectandra turbacensis	Lauraceae		X		
Schefflera actinophylla	Araliaceae	X			

2.2. Identification and Phylogenetic Analysis

Fruits were examined under a dissecting microscope for adult beetles with morphological characteristics similar to *H. hampei* (i.e., of the family Curculionidae, subfamily Scolytinae). The beetles were sorted by host plant, farm and date, and were preserved dry at −20 °C. A subsample was selected for morphological identification. Identification was based on size and morphology of bristles, surface texture of the elytra and frons, and size and color of the insect [20]. Only *Hypothenemus* is considered here. The sample size for morphological identification was determined using Cochran's sample size formula for categorical data with an alpha level of 0.01 and an acceptable margin of error of 5% [21,22].

A subsample of the scolytids identified by morphology was selected for DNA barcoding and phylogenetic analysis. DNA barcoding was used to confirm morphological identification and determine how similar the CBB population in Puerto Rico was to previously studied populations in other areas. DNA barcoding used part of cytochrome oxidase c subunit 1 (COI) mitochondrial gene, the standard for insects (http://www.barcodinglife.org/index.php/IDS_OpenIdEngine). Tissue was crushed between two glass slides, and DNA was extracted with the MoBio Ultraclean Tissue & Cells DNA Extraction kit. The primers COIF and COIR [23] were used to amplify the COI region with an annealing temperature of 55 °C. The PCR products were cleaned with ExoSAP-IT (USB Corp.) and were sequenced in both directions at the Sequencing and Genomics Facility of the University of Puerto Rico (UPR-SGF). Sequences were assembled and examined for errors using Sequencher 4.8 (Gene Codes Corp.) and compared to the GenBank database. Only COI sequences with >98% similarity were accepted as confirmed identifications [24].

For phylogenetic analysis, GenBank reference sequences of *H. hampei*, *H. obscurus* (Fabricius), *H. eruditus* Westwood, *H. seriatus* (Eichhoff), and unidentified *Hypothenemus* spp. were included [23,25,26]. Although a phylogeny based on only one sequence has limited resolving power, no other sequences are available for all the reference taxa of interest. The sequences were aligned using Muscle in Mega [27]. Maximum-likelihood trees were constructed using RAxML (Randomized Axelerated Maximum Likelihood) version 8.0 [28] with GTRCAT model of evolution, 25 gamma categories, and the automatic Bootstrap MRE [29,30]. The analysis was conducted on a CIPRES Science Gateway Web server (on RAxML-HPC Black box tool version 8.2.10) [31]. *Xylosandrus compactus* (Eichhoff) (Curculionidae, Scolytinae) was used as an outgroup [25] and sequences of *H. hampei* from other areas were included for comparison.

2.3. Preference and Feeding Tests

Since *G. guidonia* was one of the most abundant tree species and produced the most fruits in all farms sampled, as well as produced fruits all year long (including when coffee fruits were not present), it was selected for preference tests in the laboratory. One lab-reared, adult CBB female was placed inside a jar with a mature fruit of *G. guidonia* and a mature fruit of *C. arabica.* Screw-top glass jars 70 mm in diameter and 90 mm high were used, large enough that the two fruits were not touching. CBBs were placed between the two fruits. The test was run twice with 50 replicates each. Behavior was observed daily for 10 days, registering which fruit was penetrated first and if the CBB remained within that fruit. After a month, the two fruits in each jar were dissected to search for evidence of reproduction and feeding.

Alternative food sources were also tested to determine whether *H. hampei* consumed fruits other than coffee. Fruits previously reported as food sources in laboratory tests with *H. hampei* were used: *Leucaena leucocephala* (Lam.) De Wit [6] and *Cajanus cajan* [32]. Individual, lab-reared adult CBB females were placed in transparent plastic vials (40 × 100 mm, plugged with bonded dense-weave cellulose acetate) with the fruit of one of the two species. Fruits were used within 24 h of harvest. Fifty individuals of *H. hampei* were used for each species. Each CBB was observed until it died or penetrated a fruit. The day after the fruit was drilled, it was opened to see if seed tissues had been consumed. To obtain a uniform cohort for these tests, we used young female CBBs raised in an incubator at 25 °C on the artificial diet Cenibroca [33]. The tests were performed in an incubator at 25 °C and 80–96% relative humidity.

2.4. Association between Common Trees and CBB

To determine if some shade trees are a refuge for CBB or otherwise associated with it, we evaluated whether the presence of *I. vera* (Fabaceae-Mimosoideae) and *G. guidonia* (Meliaceae) trees in coffee farms was associated with CBB infestation at each site. *Inga vera* and *G. guidonia* were chosen for this analysis because: (i) They were present in most of the farms visited, (ii) they are the most abundant and important species of the coffee agroecosystem in Puerto Rico according to Arango [34], and (iii)

legumes (e.g., *I. vera*) were reported as alternate hosts of CBB in Central Africa by Schedl [16,18]. This analysis included a larger dataset than that used above, from 110 sites in 61 farms planted with *Coffea arabica*, sampled from August to November 2014 [10]. (Sites were defined as a collection of coffee plants of any extent; they were selected to represent the geographical, topographical, and ecological diversity of coffee farms in Puerto Rico.) At each site, the total number of fruits and bored fruits were counted for three branches each on three coffee plants. Counting three branches for three plants is standard for sampling CBB; CBB spatial distribution tends to be irregular [10]. Infestation was defined as the proportion of fruits showing CBB perforation [10,35].

The effect of presence of each species on the CBB infestation was evaluated with a generalized linear mixed model (GLMM). This model is useful when the data are hierarchically structured [36]; in this case the sites were nested within farms, and farms within municipalities. This model was used in previous CBB infestation studies [35]. CBB infestation rate by site was the response variable; the presence of plant other than coffee was used as a fixed effect. The date of sampling, municipality, farm, and site were used as random effects. Binomial error distribution was used to evaluate the effect of presence compared to absence of plants on CBB infestation. There were fifteen different possible models using the presence of plants as a fixed effect and adding and/or removing the random effects (date of sampling, municipality, farm, and site) sequentially. To determinate the best model, the Akaike Information Criterion (AIC) was applied. We used the cumulative distribution function for the standard logistic distribution on the output coefficients to present the results of infestation in a probability scale [37]. This was done with the plogis function in R 3.4.3 [38].

3. Results

3.1. Plants Evaluated in the Field

Of the eight plant species with perforated fruits, individuals of the subfamily Scolytinae were found only in *C. cajan*, *G. guidonia*, *I. vera*, and *Schefflera actinophylla* (Endl.) Harms (Table 3).

Table 3. Scolytinae and *Hypothenemus* spp. collected and identified in potential alternate host plants of the coffee berry borer in Puerto Rico. Numbers of beetles are shown.

Plant Species	Fruits Examined	Scolytinae	*Hypothenemus* Identified	*H. hampei*	*H. obscurus*	*H. eruditus*	*H. seriatus*	*H.* spp.
Guarea guidonia	1735	2663	533	12	89	29	0	403
Cajanus cajan	777	783	29	4	1	9	6	9
Inga vera	982	115	24	10	0	7	5	2
Schefflera actinophylla	100	2	1	1	0	0	0	0
Cordia sulcata	60	0	0	0	0	0	0	0
Cupania americana	50	0	0	0	0	0	0	0
Miconia serrulata	150	0	0	0	0	0	0	0
Nectandra turbacencis	40	0	0	0	0	0	0	0
Total	**3894**	**3563**	**587**	**27**	**90**	**45**	**11**	**414**

Both total Scolytinae and *H. hampei* were found in the highest numbers in April, at the beginning of the rainy season but before coffee fruits were available to infest (Figure 1). Patterns of total Scolytinae and *H. hampei* per month were generally similar except in September, when the number of total Scolytinae rose but the number of *H. hampei* did not. Reproduction of Scolytinae was only observed in three of the eight plant species examined: *C. cajan*, *G. Guidonia*, and *I. vera*. The frequency of life cycle stages (eggs, larvae, pupae, and juveniles) was less than <1% of the total number of fruits examined (Table 4). These stages were not identified because they are not included in taxonomic keys.
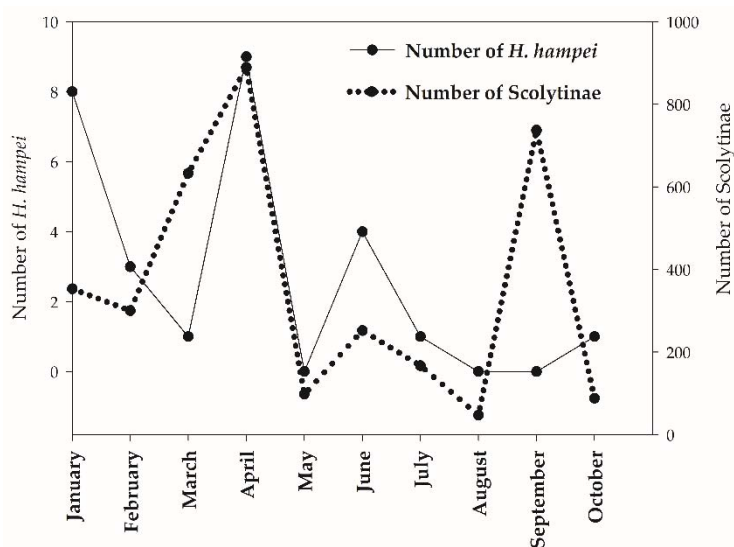
Figure 1. Number of Scolytinae beetles and *Hypothenemus hampei* found in potential alternate hosts per month. Fruits are not present from January to May; the dry season is February and March.

Table 4. Frequency of life cycle stages of scolytid beetles. Reproduction was only observed in three of the eight plant species examined. Adults were most common stage in all three species.

Host Plant	Number of Fruits Examined	Fruits with Adults	Fruits with Eggs	Fruits with Larvae	Fruits with Pupae	Fruits with ♀Juveniles	Fruits with ♂Juveniles
Cajanus cajan	777	15.8%	1.0%	2.5%	2.0%	2.5%	1.1%
Guarea guidonia	1735	37.6%	2.7%	3.3%	2.4%	2.0%	0.3%
Inga vera	982	5.2%	0.2%	0.2%	0.2%	0.3%	0.1%

3.2. Identification and Phylogenetic Analysis

A total of 3563 individuals of the subfamily Scolytinae were recorded (Table 3). Of the 587 individuals identified, 27 (4.6%) were identified as *H. hampei*; they were found in *G. guidonia* (12), *I. vera* (10), *C. cajan* (4), and *S. actinophylla* (1). These 27 individuals mostly came from samples taken between January and June 2015. In most cases, DNA barcoding agreed with identifications based on morphology. All beetles that were identified by barcoding as *H. hampei* in this study had 99% COI sequence similarity with *H. hampei* reference sequences [25], and they formed a clade with 100% bootstrap support (Figure 2). The 560 remaining individuals were identified as other species of *Hypothenemus*: 90 as *H. obscurus*, 45 as *H. eruditus*, 11 as *H. seriatus*, and 414 as unidentified *Hypothenemus* spp. (Table 3).

3.3. Preference and Feeding Tests

Ninety of the 100 female CBBs initially selected *G. guidonia* fruits over coffee fruits. Seventy CBBs that selected *G. guidonia* fruits penetrated the fruits by the second day. The remaining 10 CBBs initially selected coffee fruits; all of these, and two that originally selected *G. guidonia* fruits but did not penetrate them, drilled coffee fruits by the second day of observation (reaching the endosperm). Eventually, 76 of the 90 CBBs that first selected *G. guidonia* fruits left them to perforate coffee fruits. Fourteen CBBs remained in *G. guidonia* fruits at the end of the experiment but were dead. Reproduction was observed in 36 CBBs in coffee fruits, but reproduction and feeding were never observed in fruits of *G. guidonia*.

Feeding on the seeds of *L. leucocephala* and *C. cajan* by CBBs was not observed. In one case, a CBB penetrated a *C. cajan* seed and left scrape marks on the cotyledon, but no frass were found (which would provide evidence of feeding) and the CBB died without reproducing. The average survival of CBBs without food was 11 days.
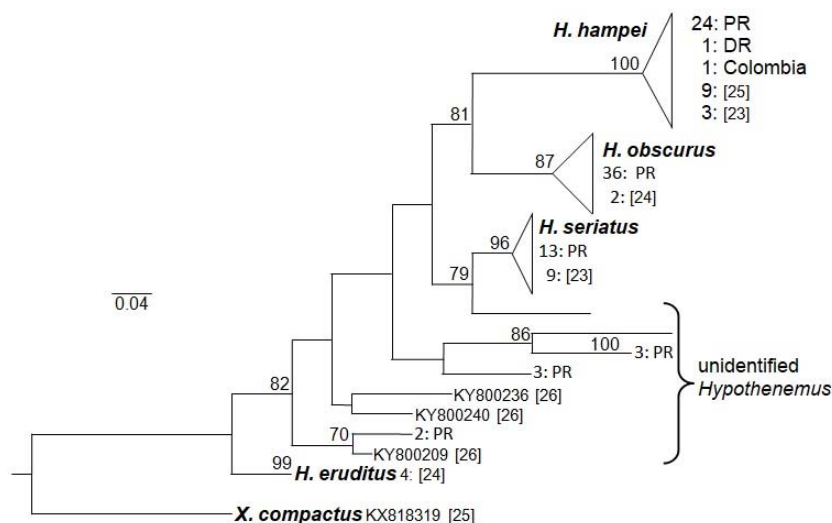
Figure 2. Maximum-likelihood tree of *Hypothenemus*. Numbers at nodes show bootstrap values over 50%. Numbers of individuals per clade and source is shown at the right. Individuals used for DNA barcoding were a subset of those identified by morphology. Sources of additional sequences: [25] Mitchell and Maddox 2010; [23] Gautier 2010; [26] Kambestad et al. 2017. GeneBank accession number for sequences generated in this study: *H. hampei*: (MK622703 to MK622727); *H. obscurus* (MK622728 to MK622760 and MK622773 to MK622775); *H. seriatus* (MK622761 to MK622772), *Hypothenemus* sp. (MK622776 to MK622788); *H. hampei* from Colombia (MK622701); and *H. hampei* from Dominican Republic (MK 622702). Other sequences used: *H. hampei*: GU133360, GU133362,GU133360 [23]; KX818256-KX616263 [25]; *H. obscurus*: KX818295-6, KX818299-300, KX818302 [25]; *H. seriatus*, KX818303-11 [25]; *H. eruditus*, KX818248-51 [25]; and *Hypothenemus* spp., KY800209, KY800236, KY800240 [26]. The tree was rooted to the outgroup *Xylosandrus compactus*, KX818319 [25]. Origin of samples: PR: Puerto Rico; DR: Dominican Republic.

3.4. Association between Common Trees and CBB

The presence of the two most common trees on coffee farms was not significantly associated with CBB infestation (Table 5). The probability of infestation of coffee fruits was 9.5% in the presence of *I. vera* and 13.5% in its absence, but this difference was not significant. The best model (selected by AIC) included date, farm, municipality, and site as random effects. Similarly, the probability of infestation was 9% in the presence of *G. guidonia* and 14% in its absence, but the difference was not significant; the best model selected included date, farm, and site as random effects.

Table 5. Generalized linear mixed model (GLMM) estimates of the association between infestation rate of coffee and presence of *Inga vera* and *Guarea guidonia* in coffee farms.

Species	Estimated Intercept	Estimated Coefficient	Z	P
Inga vera	−1.8545	−0.3976	−0.92	0.36
Guarea guidonia	−1.7836	−0.5183	−1.02	0.31

4. Discussion

Can plants other than coffee serve as alternate hosts for *H. hampei*? The use of alternate hosts in the coffee farms of Puerto Rico was infrequent compared to infestation rates in coffee [10]: Only 27 individuals of *H. hampei* were found in 3894 fruits examined. Scolytid eggs, larvae, pupae, and juveniles were infrequent in the fruits of potential alternate hosts. Observations of *H. hampei* in fruits of alternate hosts were mainly during the season in which no coffee fruits were present, or were too immature to be penetrated. This suggests that the lack of coffee fruits may trigger this behavior. However, the fact that one individual of *H. hampei* was found on an alternate host during the coffee

fruiting season indicates that there may be other factors that cause *H. hampei* to penetrate alternate hosts. This is also the case with *H. obscurus*, which occasionally attacks coffee fruits in Hawaii despite having a preference for macadamia [39]. In lab tests, most CBBs initially penetrated *G. guidonia* fruits instead of coffee fruits, a surprising finding given that its preferred host is coffee, but almost all later moved to coffee fruits. It would be interesting to explore volatile compounds produced by *G. guidonia* fruits and their potential use as lures for the CBB.

Inga vera and *G. guidonia* were the alternate hosts in which the greatest number of *H. hampei* individuals were found, and there was a lower probability of infestation of coffee fruits when these species were present, although the effect was not significant. It is possible that these species grow better under certain environmental conditions which are less conducive to CBB infestation, and there is no direct relationship with CBB. However, it is also possible that these trees attract some *H. hampei* individuals who bore them and die without ever attacking coffee fruits. Further work on this relationship is needed. It might confer an added value to these species, not only as shade trees, but also as a tool in pest management.

In preference tests, fruits of *G. guidonia* were initially drilled, but only occupied for a short time before *H. hampei* moved to coffee fruits. It can thus be inferred that these fruits only served as temporary shelter. This behavior could explain the reports of individuals of *H. hampei* on plants other than coffee in several coffee-producing countries [6,12,16,40].

Although a single CBB penetrated and gnawed a *C. cajan* seed, there was no evidence of feeding. These results contrast with experiments using *C. cajan* seeds as an alternative food to maintain colonies of *H. hampei* in Guatemala [32]. In those experiments, normal development of all stages was observed on this plant, supporting the conclusion that the seeds of *C. cajan* meet the nutritional requirements of *H. hampei*. Similarly, the seeds of *L. leucocephala*, which were not used by *H. hampei* in our laboratory tests, were reported as a food source in laboratory experiments in Philippines [6]. These authors reported that another 21 species of plants, distributed in nine families were also used by *H. hampei* as food in the laboratory. In contrast, our laboratory results appear to agree with previous claims that *H. hampei* is monophagous [41].

These reports of polyphagous behavior of *H. hampei* might reflect the phenotypic plasticity of many species of the genus *Hypothenemus*, which allows them to use varied sources of food including different species and even decomposing material [7]. Many species have developed specialized feeding behaviors (e.g., host specificity) to reduce competition for food [42–44]. However, these behaviors can complicate survival when the food source is scarce [43,45]. Phenotypic plasticity may allow the more flexible individuals to use alternative resources [46].

For example, *H. obscurus* mainly attacks macadamia in Hawaii, but occasionally damages coffee fruits and coffee branches [39]. In certain cases, specialized species can maintain genotypic characteristics that allow them to take advantage of ancestral hosts [47]. Moreover, it is possible that differences in monophagy compared to polyphagy might reflect variation among beetles identified as *H. hampei*, either because beetles were misidentified in some studies, because there may be cryptic species that differ in behavior, or because some populations may have retained more phenotypic plasticity than others.

In this study, we found 3563 Scolytinae beetles in potential alternate hosts. Likewise, in a previous study many Scolytinae were found in fruits of several of the most important plants associated with coffee farms in Puerto Rico [34]. This is why the task of finding *H. hampei* individuals in fruits of alternate hosts is similar to searching for a needle in a haystack, and suggests that some of the beetles identified as *H. hampei* in previous studies may have belonged to other species. Similarly, a recent survey of >18,000 herbarium specimens of potential alternate hosts from coffee-growing regions of Africa found *H. hampei* in fruits of several species of *Coffea*, but not in any other plants [19].

The most abundant species identified here was *H. obscurus* followed by *H. eruditus*, which are among the 16 species of *Hypothenemus* in Puerto Rico [20]. *H. obscurus* is the most economically important *Hypothenemus* after *H. hampei*, as it may affect the seeds of many other crops [20,39].

In addition, *H. obscurus* and *H. eruditus* have been reported feeding on the coffee mesocarp [7,39], which may imply additional damage to the fruit, since they can facilitate entry of pathogens [48,49].

DNA barcoding was used to support morphological identifications of *H. hampei*. The *H. hampei* clade based on COI sequences was well-defined and had 100% bootstrap support (Figure 2). Previous studies on possible alternate hosts of the *H. hampei* did not use DNA barcoding to confirm identifications, a limitation considering the large number of similar species in *Hypothenemus*. In some cases, barcoding data did not support morphological identifications because the COI sequences had no hits >95% in GenBank (Figure 2). These individuals could be new species or species not represented in GenBank. The unidentified *Hypothenemus* in this study were variable in the COI region sequenced, but none was closely related to *H. hampei*. The COI phylogenetic tree (Figure 2) differed from a previous study in the relationship among *H. hampei*, *H. obscurus*, *H. seriatus,* and *H. eruditus* [25], but neither had strong bootstrap support at all the relevant nodes. This discrepancy, together with unidentified sequences mentioned above, the economic importance and the ubiquity of *Hypothenemus*, shows that more phylogenetic studies on this group are needed.

5. Conclusions

This study shows that *H. hampei* in Puerto Rico can occasionally use fruits of plant species other than coffee as a shelter, but there is no evidence that alternate hosts are used as a source of food or place of reproduction. Although our results are in agreement with the previous general observation on the monophagy of *H. hampei*, there are reports of polyphagy of this species in other coffee-producing countries (e.g., Guatemala and the Philippines), which highlights the importance of the study of alternative hosts of *H. hampei* and their implications for the management of this pest in different locations along the coffee production belt.

This study shows that alternate hosts are most likely not a significant source of re-infestation of plots after the dry period. Consequently, and taking into account the life history of the insect, the presence of the CBB might be significantly reduced by the management of the remnant coffee fruits in the plants after harvest [10], as well as the elimination of abandoned coffee farms which serve as a reservoir of CBB between crops. Of course, it is possible that CBB have other refugia that have not yet been found or studied.

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