Conservation biocontrol of the coffee berry borer in coffee farms through ant predation

By

Jannice Newson

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science (Conservation Ecology)
School for Environment and Sustainability
University of Michigan, Ann Arbor
May 1st, 2020

Masters Committee:

Professor Ivette Perfecto, Primary Advisor
Professor John Vandermeer, Secondary Advisor

Acknowledgements

Firstly, I would like to thank Ivette and John for supporting me through the process of field work and completion of this project. I thank Zachary Hajian-Forooshani and Chatura Vaidya for their assistance in my project development. I thank all other members of Ivette Perfecto's and John Vandermeer's lab, staff at Gran Batey and la Finca de Raul Toledo, and the field work team in Puerto Rico. I thank Meaghan Pearson for her continued friendship and support throughout the course of this project. This work was supported by the Rackham Graduate Student Research Grant, the Alpha Kappa Alpha Sorority, Inc. Fantasy in Pink Scholarship, and the School of Environment and Sustainability Research Grant. Lastly, and most importantly, I would like to thank my mother Brenda Paige for being a source of love and comfort person to me through this entire process.

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Abstract

Pesticides threaten biodiversity in farms and the any matrices where they are used. In the coffee system, conservation biocontrol is a sustainable agriculture practice that has the potential to ameliorate the effects of pesticide by encouraging the presence of natural enemies. The coffee berry borer (CBB) remains the most important insect pest in coffee around the world. Spending the majority of its life cycle within coffee berries, CBB evades topical applications of pesticide. Ants have been shown to be important predators of the coffee berry borer in field and laboratory settings. We measured CBB damage in low, medium and high coffee bush branches, and assessed the ability of ants to prey on adult CBB before and after entering berries in coffee farms in Puerto Rico. This was done by assessing the proportion of damaged berries and survival of adult beetles within the berries at the time of sampling. Over 20,000 coffee berries were assessed from 220 plants in two farms in Puerto Rico. Ant identity and activity was determined via tuna fish baits in individual coffee bushes. Fifteen ant species were observed within the two farms, but only five species were abundant enough to assess their impact on the CBB. Top branches had significantly less damage than medium and low branches. 73% of the plants surveyed had some level of CBB damage, and average damage on a per plant basis was 6.4%. Of those berries with damage, 41% had live adult beetles inside. Plants with *W. auropunctata* and *Solenopsis invicta* had lower CBB damage than plants where these species were not present, although for *W. auropunctata* this effect was significant only for one of the farms. Contrary to expectations, plants with *Pheidole moerens* and *Monomorium floricola* had significantly more CBB damage than plants without these species. With respect to survival (adult CBB that were alive and inside the berries at the time of sampling), CBB had a significantly lower survival in plants with *W. auropunctata*, while it has a significantly higher survival in plants with *S. invicta*. Our results suggest that the effect of ants on CBB damage and survival is highly contextual depending on the ant species that occupy the coffee plants. Nonetheless, this study identified two species that had a significantly negative effect on damage by the CBB in coffee plants, *S. invicta* and *W. auropunctata*, but only *W. auropunctata* also had a negative effect on the survival of the CBB adults once they penetrated the berries. Understanding the distribution of ant species within farms can be important for implementing conservation biocontrol of the CBB.

Keywords: sustainable agriculture, Formicidae, Puerto Rico, *Wasmannia auropunctata*, *Solenopsis invicta*, *Monomorium floricola*, *Pheidole moerens*

Introduction

Agriculture occupies 40% of land on Earth, making it the prevailing use for all arable land (Ellis et al. 2010). Variability in climate, crop varieties, soil conditions, soil management, biodiversity, and many other factors in the agro-ecosystem impact crop yields and ultimately the lives of farmers and all of us reliant on farming for a source of nutrition. A major problem inherent to many agro-ecosystems is pest outbreaks. Intensification of agriculture to chemically reliant monocultures results in a loss of biodiversity (Matson et al. 1997; Perfecto et al. 1997; Armbrecht et al. 2005; Landis 2017). This loss of biodiversity includes loss of natural enemies that provide the important ecosystem service of pest control (Wilby and Thomas 2002). Pest outbreaks are only expected to worsen when considering climate change. Although responses of insects to climate change are context dependent and complex, studies have consistently shown earlier flight periods, enhanced winter survival and faster development rates (Porter et al. 1991; Ladányi and Horváth 2010; Robinet and Roques 2010; Juroszek and Von Tiedemann 2013)

Coffee agro-ecosystem

The coffee agro-ecosystem is one of particular interest, as it provides a commodity that is exported worldwide and serves as the livelihood for at least 25 million small scale farmers (Pendergast 1999; Perfecto and Vandermeer 2015). Two commercial species exist: *Coffea* arabica and *Coffea canephora*. The cultivation of coffee exists on a spectrum between sun coffee (or coffee monoculture) and rustic shade coffee. Sun coffee is characterized by a plantation devoid of trees, with coffee plantings only, while shade coffee is interspersed with trees and other plant species. The term 'shade coffee' is not uniform; shade coffee ranges from forest-like assemblages where only the lower strata is removed to cultivate coffee to monospecific, heavily pruned commercial trees (Moguel and Toledo 1999). The coffee agro-ecosystem has seen a shift to sun coffee since the 1990s in order to increase short term yields (Perfecto et al. 1996). This shift has left concerns for biodiversity in the tropical regions where coffee is grows, as shade coffee has been found to boast biodiversity similar to that of intact forest (Perfecto et al. 1996, 2005; Borkhataria et al. 2012).

Pests in the coffee agro-ecosystem

The coffee agro-ecosystem also suffers from crop-damaging pests. There are four major pests in coffee in Puerto Rico: coffee leaf rust (*Hemileia vastatrix*), the green coffee scale (*Coccus viridis*), the coffee leaf miner (*Leucoptera coffeella*), and the coffee berry borer (*Hypothenemus hampei*) (Vandermeer et al. 2010). Despite the potential damage that can be caused by these pests, in some coffee systems, a complex set of interactions between the pests and other members of the food web prevent devastating outbreaks. In coffee systems in Mexico and other neotropical regions, the ant *Azteca sericeasur* is a driving force in creating autonomous pest control through multiple direct and indirect interactions with pests and natural enemies (Vandermeer et al. 2010; Perfecto et al. 2014; Vandermeer and Perfecto 2019).

Hypothenemus hampei, Curculionidae: coffee berry borer

Among coffee pests, the coffee berry borer (CBB) is the most important insect pest, as it accounts for $500 million in losses each year globally (Vega et al. 2009). The CBB is originally from Africa, a specialist on coffee, and carries out most of its life cycle within coffee fruits. Although it does not cause damage to the coffee leaves or stems, the boring into the coffee fruit results in reduction of the quality and quantity of yield, vulnerability to additional pests and disease, and premature senescence of green coffee berries (Moore and Prior 1988; Damon 2000). Female beetles colonize coffee fruits, as the males have degenerate wings and are not able to move from fruit to fruit (Damon 2000). A proteobacteria, *Wolbachia* spp, skews the sex ratios of CBB offspring towards females, which allows for the pest to spread *en masse*

(Brun et al. 1995). The female beetle bores into the fruit, lays eggs, and the growth and feeding of the larvae eventually destroy the fruit. Matting among siblings takes place inside the berry. The emergence of the new fertilized females is triggered by increased temperature and relative humidity, and the process restarts (Jaramillo et al. 2006).

Controlling the coffee berry borer

The life cycle of the CBB occurs almost entirely confined within coffee fruits, making it difficult to utilize pesticide for control. Nevertheless, endosulfan and chlorpyrifos are topically applied in an attempt to combat CBB. Both of these pesticides pose some human and environmental health risks (Jaramillo et al. 2006). CBB has high levels of inbreeding, so resistance to endosulfan has occurred in some areas, but no such resistance has been found for chlorpyrifos yet (Brun et al. 1989, 1994; Jaramillo et al. 2006). Another simple approach is cultural control, which consists of removing old berries post-harvest to prevent CBB spread (Aristizábal et al. 2017). Cultural control also reduces parasite populations that can control CBB, however removal of CBB is prioritized over parasite preservation (Damon 2000).

Biocontrol is another way to control CBB. Classical biocontrol involves introducing, rearing and mass releasing a species from the native region of the pest to the region where it is causing problems. Classical biocontrol has been used to combat CBB using mass reared parasitoid wasps, fungi, and nematodes (Jaramillo et al. 2006). Risks for classical biocontrol include introduction of invasive species and competition with native species (Escobar Ramírez et al. 2019). Conservation biocontrol is another form of biocontrol that advocates for conserving natural enemy habitat on farms to protect predators and buffer against pest outbreaks. The shift to chemically driven sun coffee has diminished natural enemy populations (Perfecto et al. 1997; Armbrecht and Gallego 2007; Landis 2017), which leaves pests unchecked, resulting in the need for pesticides. However, conserving habitats for natural enemies can allow farmers to benefit from the ecosystem service of pest control provided by biodiversity (Wilby and Thomas 2002; Geiger et al. 2010).

Ants have been found to predate on CBB in field and lab settings (Trible and Carroll 2014; Morris and Perfecto 2016; Morris et al. 2018). Studies have found more diverse ant communities in shade coffee than in sun coffee, and ants are more efficient at removing CBB in shade coffee than in sun coffee (Armbrecht and Perfecto 2003; Armbrecht et al. 2005; Armbrecht and Gallego 2007; Mariño et al. 2016; Morris et al. 2018). Multiple ant species predate CBB, including the following: *Azteca sericeasur, Solenopsis geminata, Pseudomyrmex simplex, Pheidole synanthropica, Pseudomyrmex ejectus, Procryptocerus hylaeus, Dolichoderus thoracicus, Crematogaster curvispinosa, Wasmannia auropunctata, Solenopsis picea, Solenopsis invicta,* and others (Jaramillo et al. 2006; Morris et al. 2018). Ants can predate CBB pre-berry penetration, during berry penetration, or post berry penetration.

Coffee berry borer in Puerto Rican coffee-agroecosystems

Coffee in Puerto Rico is the fifth most valuable crop and is grown in the Cordillera mountain region (Flores 2011). CBB was first reported in Puerto Rico in 2007 (NAPPO 2007), and has had devastating effects, with rates of infestation at 20% or more for 40% of the coffee region (Mariño et al. 2017). Serious levels of CBB damage can be attributed to a few things, including lack of labor force for cultural control and very little use of other pest management strategies (Mariño et al. 2016, 2017; Aristizábal et al. 2017). In addition to a lack of pest management, Hurricane Maria and Hurricane Irma destroyed 80 of commercial coffee and 90% of infrastructure in Puerto Rico (Aristizábal et al. 2017), leaving farmers to struggle in order to recover their coffee farms from weeds and vine coverage that became problematic after the hurricane reduced shade level by an average of 37% (Perfecto et al. 2019). During this period, insect pest control became a secondary problem to vine removal, and cleaning debris from the coffee farms. Income from coffee farming is low, and adoption of integrated pest management strategies is associated with higher socioeconomic status and education levels, so farmers may not be prioritizing pest control as much due to these reasons (Chaves and Riley 2001; Philpott and Dietschtt 2003).

The role of ants in biocontrol in Puerto Rican coffee farms is not entirely understood. There are few truly arboreal nesting ants in Puerto Rico, which has been shown to be important in CBB biological control in other regions (Vandermeer and Perfecto 2006; Larsen and Philpott 2010; Gonthier et al. 2013; Morris et al. 2018), and few species that forage on coffee plants in general. One study looking at ants in Puerto Rican coffee farms found that, on average, 40% of trees on coffee farms were occupied by *W. auropunctata* (Yitbarek et al. 2017). Another study that surveyed ants on coffee plants in 25 farms distributed throughout the coffee-growing region of Puerto Rico reported a total of 21 species foraging on coffee farms in Puerto Rico, with *W. auropunctata, Solenopsis invicta, Monomorium floricola,* and *Tapinoma melanocephala* being the dominant ones (Perfecto and Vandermeer, *in review*). Given the need for pest control in Puerto Rican coffee farms and the lack of active and systematic pest management strategies, it is worthwhile to assess the potential for ants for the control CBB.

Research questions

This study aims to understand the role of ants in the control or deterrence of CBB as a type of conservation biocontrol. We also are interested in how CBB attacks impact coffee seeds, as not all CBB damage results in the destruction of the seed, making the seed still viable for sale. Specifically, we investigated the following questions: (1) What is the effect of individual ant species on CBB damage and survival inside the berries? (2) How does ant species richness affect CBB attack on berries and survival of the CBB inside the berries? (3) How does CBB attack and survival vary between coffee farms and among different parts (low, medium, and high branches) of the coffee plants?

Methods:

Study area

This study took place in the Utuado municipality in the western mountainous region of Puerto Rico. Two farms, Finca Cítricos Inc. (owned by Raul Toledo) and Finca Gran Batey (own by Bernardo Morales and Lotty Aymat), were included in the study. Both farms are less than 20 hectares and have coffee interspersed with other species, such as citrus trees, banana and plantain trees, and breadfruit trees. A previous study conducted in the same farms resulted in maps of the distribution of ant species on coffee plants in an area of 2500 m^2 in Finca Gran Batey and 1950 m^2 in Finca Cítricos, Inc. (Figure 1; Perfecto and Vandermeer, *in review*). *Wasmannia auropunctata*, the little fire ant, and *Solenopsis invicta,* the red imported fire ant, were the ants with the highest levels of activity and occupancy (number of coffee plants occupied by this species) in Finca Gran Batey and Finca Cítricos, Inc., respectively. Other ant species with relatively high occupancy were *Pheidole moerens*, *Brachymyrmex heeri*, and *Monomorium floricola*.

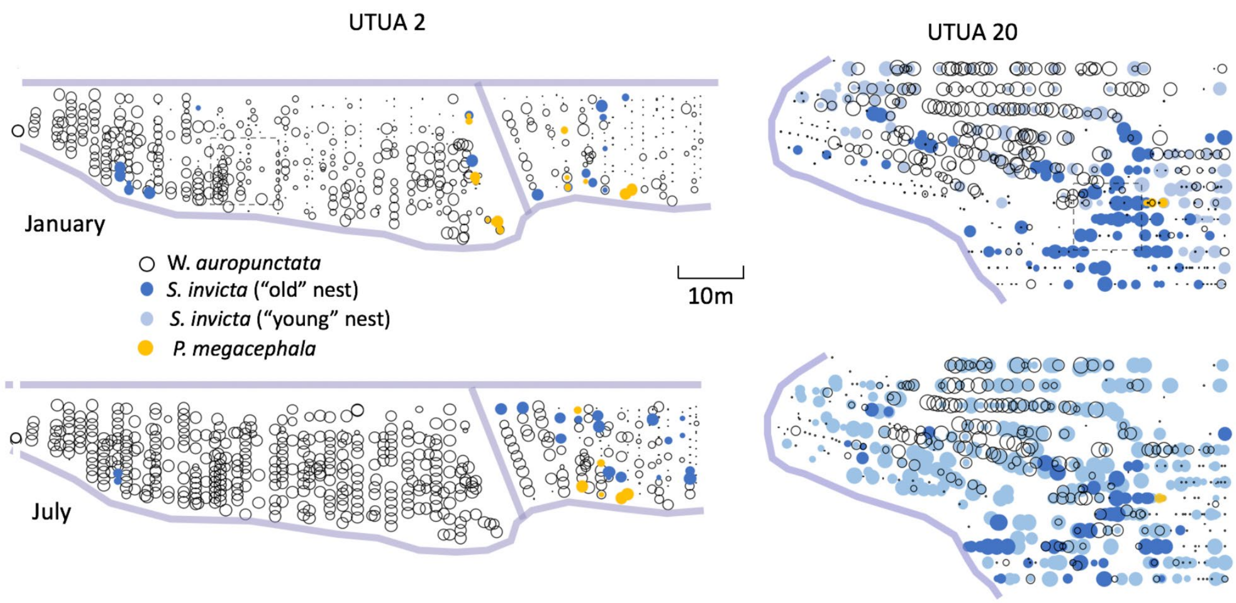

Figure 1: Map of Finca Gran Batey (UTUA 2) and Finca Cítricos, Inc. (UTUA 20) and their dominant ant distributions in 2019

Field methods

Using the two maps of the distribution of ant species on coffee plants, subsamples were made by numbering coffee plants within a section of the mapped plots. Numbered plants were selected using a random number generator. Each coffee plant selected was sampled by randomly selecting three branches originating from a main stem. Branches were classified as low, medium, or high, based on their location on the main stem. For each branch, the total number of berries and number of CBB-bored (damaged) berries were counted. CBB-bored berries were collected in plastic bags for further examination in the laboratory. Coffee plants with little to no berries, plants with berries out of reach, and berries less than two centimeters in length were excluded from the data. A total of over 20,000 berries and 220 coffee plants were assessed in total.

Laboratory methods

Collected berries were cut cross-sectionally to categorize the contents. Berries were observed with a magnifying glass and categorized based on their contents. Categories are as follows: adult CBB present or adult CBB not present. Following opening berries and classifying them, berries and their contents were frozen for four hours and then discarded outdoors.

Statistical Analyses

Statistical analysis was completed in RStudio and Microsoft Excel. CBB positionality preference (low, medium or high branches) was determined with an Analysis of Variance (ANOVA) and Tukey Honestly Significant Difference (Tukey HSD) tests. Berries bored by CBB (referred to as "damage") and berries with CBB live adults found inside the berries (referred to as "survival") were analyzed as proportions. Bored berries as a subset of total berries is "percent CBB damage", and berries with surviving adult CBB as a subset of total damaged berries is "percent CBB survival". The five most abundant ant species were used for separate analyses of the effect of ant species on the CBB. The number of species of ants in each coffee plant was used as ant species richness index to examine the effects of ant richness on CBB. Species richness analysis was completed using a quasi-poisson generalized linear model (GLM).

The effect of individual ant species on CBB damage and survival was analyzed by comparing damage and survival proportions on plants with a particular species to plants without that species using random resampling with 1000 resamples. All analyses were done for data pooled from both farms and for individual farms.

Results

Prevalence of the coffee berry borer

Cumulatively, 73% of the coffee plants had some level of CBB damage, while 41% of plants had some level of CBB survival (Table S1). Pooled data for both farms shows average CBB damage per plant at 6.4% (± 8.1) and average CBB survival at 23% (±32.5) per plant. In Finca Gran Batey, average CBB damage was 8.6% (±9.5) and average CBB survival was 16.9% (±23.3). In Finca Cítricos, Inc., average CBB damage was 4.4% (±7.2) and average CBB survival was 23.0% (±31.6). Average CBB damage is significantly higher in Finca Gran Batey than in Finca Cítricos, Inc. ($p < 0.05$), however average CBB survival between the two farms was not significantly different. CBB damage was significantly lower in the upper coffee branches than in the lower two branches overall and within both farms (Table 1).

Table 1: Coffee berry borer damage by the branch (values with different letter demarcation are statistically significant difference at p<0.05; comparisons within row only)

	Upper branch	Middle branch	Lower branch
Overall	4% ^{A}	8% ^{B}	9% ^{B}
Finca Gran Batey	5% ^{D}	10% ^{E}	9% ^{EF}
Finca Cítricos Inc.	2% ^{GH}	4% ^{H}	10% ^{I}

Ant community

15 species of ants were observed in both farms, with more species found in Finca Gran Batey (13 species) than in Finca Cítricos, Inc. (8 species) (Table 2). The five most abundant species were, *Wasmannia auropunctata*, *Solenopsis invicta*, *Monomorium floricola*, *Pheidole moerens*, and *Brachymyrmex heeri*, however *P. moerens* was not observed in Finca Cítricos, Inc. Reported results are for these five most prevalent ant species. The species richness per plant or species richness per farm did not prove to be a significant variable impacting damage or survival (Figure 3 & 4).

Table 2: Ant prevalence in Finca Gran Batey and Finca Cítricos, Inc. (bolded species received additional analysis; bolded values indicate statistical significance between the two farms at p<0.05)

Ant Species	Finca Gran Batey	Finca Citricos, Inc.
Brachymyrmex heeri	17%	6%
Brachymyrmex obscurior	5%	-
Dolichoderine sp.	1%	-
Monomorium floricola	9%	11%
Nylanderia pubens	4%	1%
Nylanderia stenheili	-	1%
Patrechina longicornis	13%	-
Pheidole exigua	1%	-
Pheidole megacephala	2%	-
Pheidole moerens	13%	-
Pheidole sp.	1%	1%
Solenopsis invicta	9%	79%
Solenopsis sp.	3%	-
Tapinoma melanocephala	-	5%
Wasmannia auropunctata	81%	31%
Overall number of species	**13**	**8**

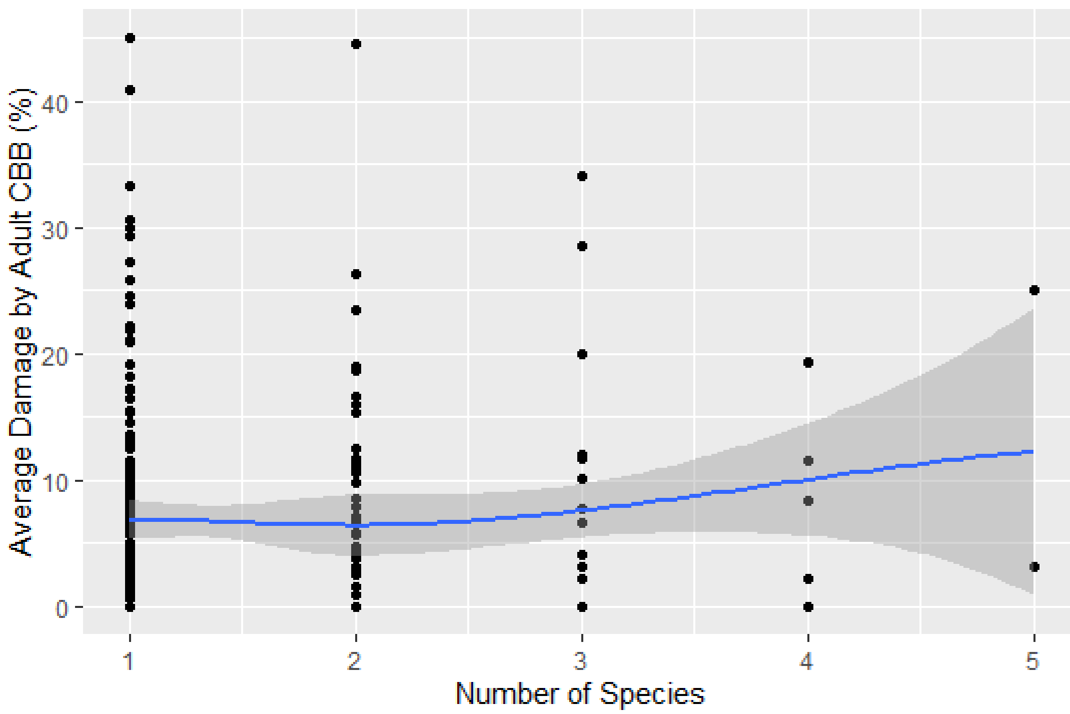

Figure 2: Effect of species richness on coffee berry borer damage

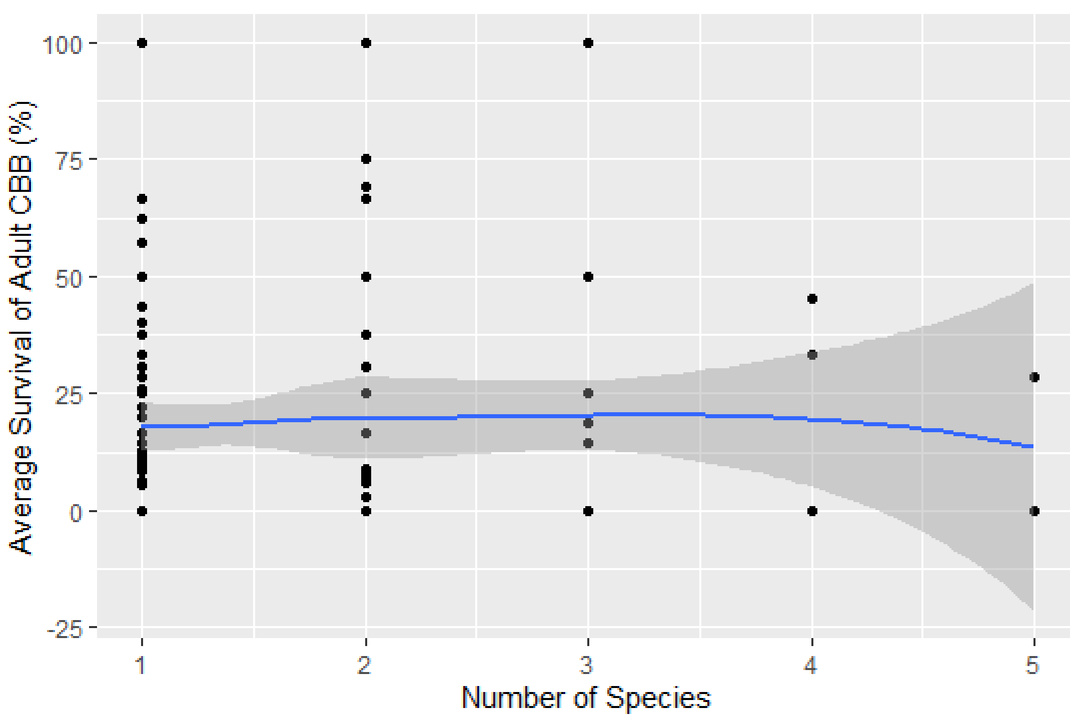

Figure 3: Effect of species richness on coffee berry borer survival

Wasmannia auropunctata

Overall (the two farms pooled together), the presence of *W. auropunctata* had no significant effect on CBB damage (Table 3; Figures 4a & 4b), but it significantly reduces CBB survival (Table 3; Figure 5). In Finca Gran Batey, *W. auropunctata* significantly reduces CBB damage when measured on a per branch basis (Table 4; Figure 6a) and CBB survival (Table 4; Figure 7). *W. auropunctata's* effect on CBB damage and survival in Finca Cítricos, Inc. is not significant, despite following the same trend as that in Finca Gran Batey (Table 5; Figures. 8 & 9).

Table 3: Overall effects of individual ant species on percent CBB damage to coffee berries and percent adult CBB survival (bolded values are significantly different at p<0.05)

	Damage – branch level		Damage – plant level		Survival – plant level	
	Present	Absent	Present	Absent	Present	Absent
W. auropunctata	6.5%	7.4%	7.5%	6.2%	**14.9%**	**25.3%**
P. moerens	**11.1**	**6.5%**	**13.1%**	**6.6%**	27.2%	17.8%
M. floricola	**10.8%**	**6.4%**	7.9%	6.9%	25.6%	17.8%
B. heeri	7.7%	6.7%	8.3%	6.9%	22.0%	18.1%
S. invicta	5.6%	7.4%	**5.0%**	**8.1%**	23.8%	15.6%

Table 4: Farm level effects of individual ant species on proportion CBB damage to coffee berries and adult CBB survival in Finca Gran Batey (bolded values are significantly different at p<0.05)

	Damage – branch level		Damage – plant level		Survival – plant level	
	Present	Absent	Present	Absent	Present	Absent
W. auropunctata	**7.1%**	**12.4%**	8.4%	9.6%	**13.7%**	**34.2%**
P. moerens	11.1%	7.6%	**13.1%**	**8.0%**	27.2%	15.4%
M. floricola	**12.8%**	**7.6%**	9.7%	8.5%	24.6%	16.2%
B. heeri	9.0%	7.8%	9.3%	8.5%	20.7%	16.2%
S. invicta	9.2%	7.9%	8.3%	8.7%	**33.6%**	**15.3%**

Table 5: Farm level effects of individual ant species on proportion CBB damage to coffee berries and adult CBB survival in Finca Cítricos, Inc. (bolded values are significantly different at p<0.05)

	Damage – branch level		Damage – plant level		Survival – plant level	
	Present	Absent	Present	Absent	Present	Absent
W. auropunctata	3.9%	5.3%	3.5%	4.8%	20.7%	21.8%
P. moerens	-	-	-	-	-	-
M. floricola	8.2%	4.4%	5.6%	4.3%	27.0%	20.7%
B. heeri	2.0%	5.0%	3.5%	4.5%	27.3%	21.0%
S. invicta	5.8%	3.6%	4.6%	3.7%	21.8%	20.0%

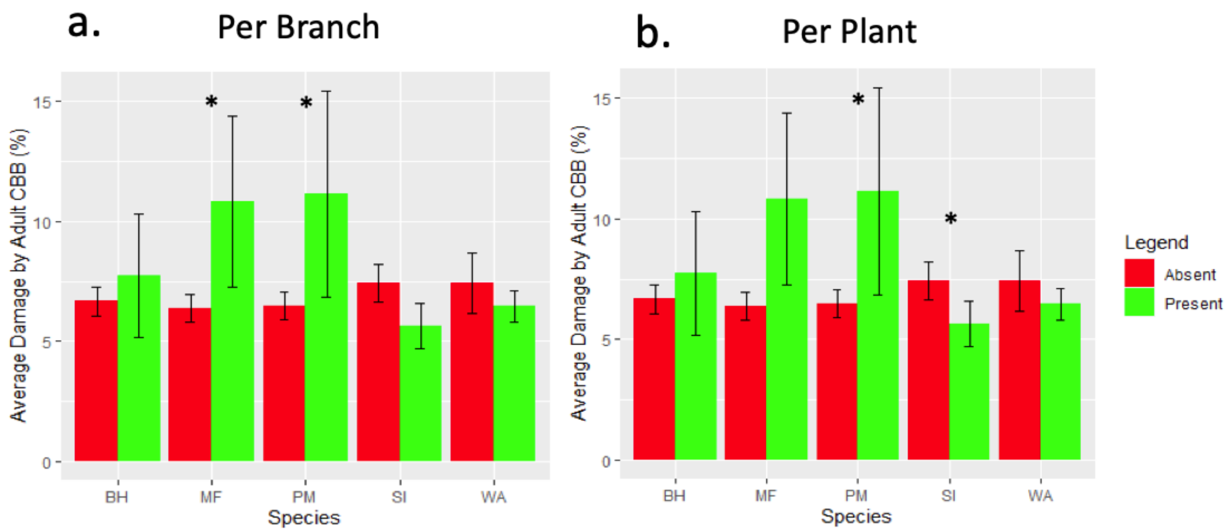

Figure 4: Effect of presence and absence of individual ant species on average damage inflicted by adult CBB; a) per branch basis, b) per plant basis; BH = B. heeri, MF = M. floricola, PM = P moerens, SI = S. invicta, and WA = W. auropunctata).

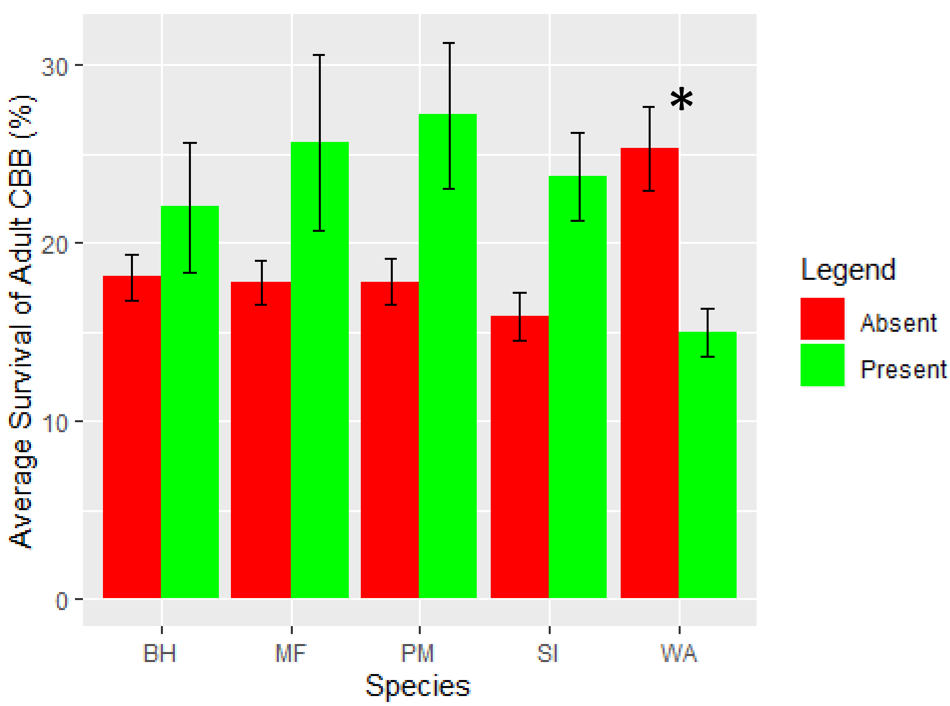

Figure 5: Effect of presence and absence of individual ant species on average survival of adult CBB (BH = B. heeri, MF = M. floricola, PM = P moerens, SI = S. invicta, and WA = W. auropunctata).

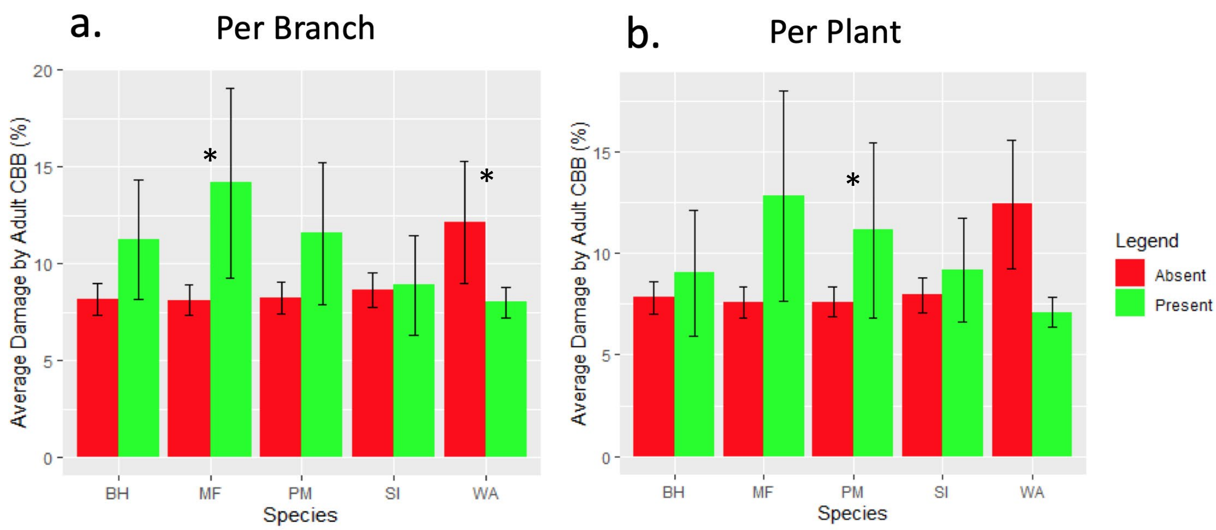

Figure 6: Effect of presence and absence of individual ant species on average damage inflicted by adult CBB in Finca Gran Batey; a) per branch basis, b) per plant basis; BH = B. heeri, MF = M. floricola, PM = P moerens, SI = S. invicta, and WA = W. auropunctata).

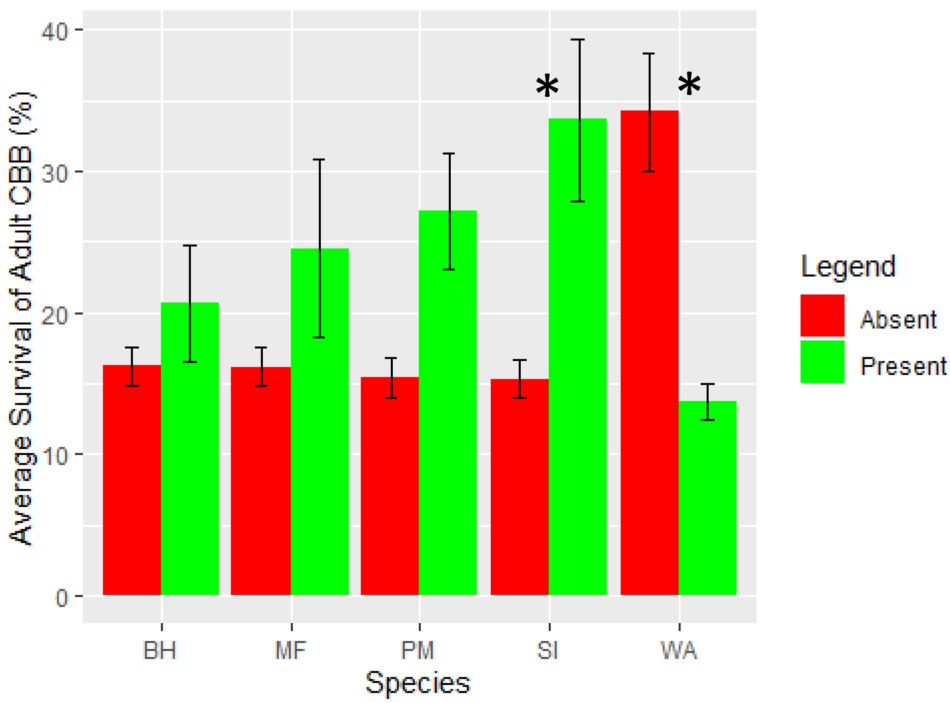

Figure 7: Effect of presence and absence of individual ant species on average survival of adult CBB in Finca Gran Batey (BH = B. heeri, MF = M. floricola, PM = P moerens, SI = S. invicta, and WA = W. auropunctata).

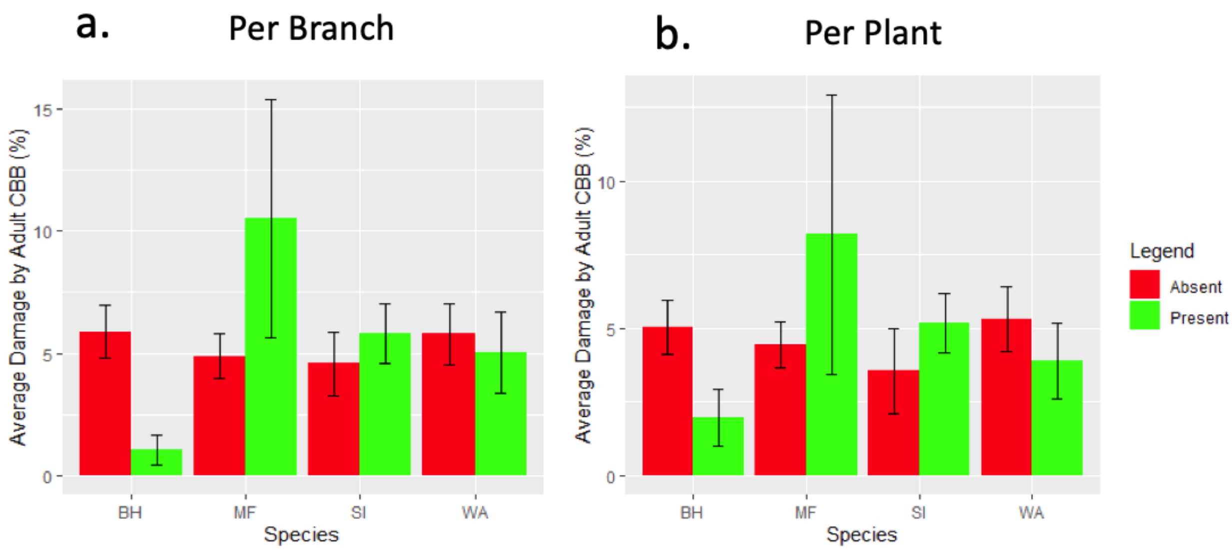

Figure 8: Effect of presence and absence of individual ant species on average damage inflicted by adult CBB in Finca Cítricos, Inc.; a) per branch basis, b) per plant basis; BH = B. heeri, MF = M. floricola, SI = S. invicta, and WA = W. auropunctata).

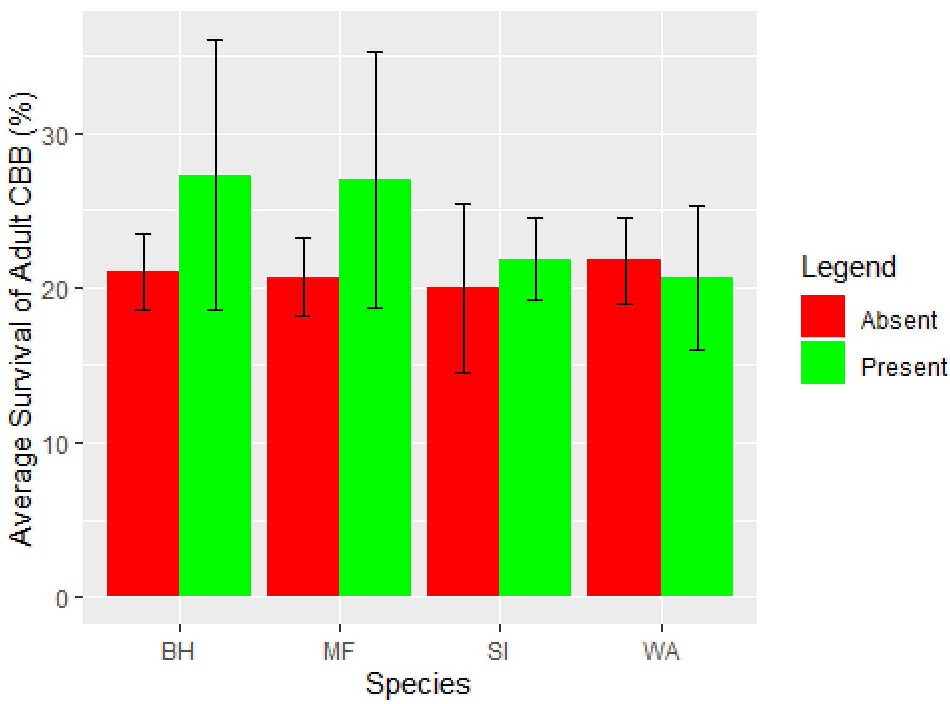

Figure 9: Effect of presence and absence of individual ant species on average survival of adult CBB in Finca Cítricos, Inc. (BH = B. heeri, MF = M. floricola, SI = S. invicta, and WA = W. auropunctata).

Solenopsis invicta

Overall, *S. invicta* significantly reduces CBB damage when measured at the plant level (Table 3; Figure 4b) but this significance is lost when damage was considered at the per branch level (Table 3; Figure 4a). *S. invicta* also increases CBB survival, however this trend is only significant in Finca Gran Batey (Table 4 & 5; Figures 7 & 9).

Pheidole moerens, Monomorium floricola, and Brachymyrmex heeri

P. moerens significantly increases CBB damage at the branch and plant level (Table 3; Figures 4a & 4b). *P. moerens* is not present in Finca Cítricos, Inc., but in examining Finca Gran Batey alone, *P. moerens* also significantly increases CBB damage at the plant level (Table 4; Figure 6b). *M. floricola* also significantly increases CBB damage at the branch level when considering the pooled data for both farms and in Finca Gran Batey alone (Table 3 & 4; Figures 4a & 6a). *B. heeri*, the least abundant of the five species analyzed, gives no significant results for its impact on CBB damage or survival (Table 3-5; Figures 4-9).

Discussion:

Vertical distribution of CBB damage

We found that higher branches suffer less CBB damage than lower branches. Since we lack information on the distribution of the ants within plants, we cannot attribute this effect to the ants. However, since all ants included in this study nest either on the ground or at the base of the coffee tree, the activity of ants tend to be higher in lower branches than in the upper branches. Based on many years of experience sampling ants in coffee plants, Perfecto (personal communication) noticed that there are less ants foraging on the upper branches. Therefore, ants are not good candidates to explain the lower levels of damage to upper branches. Anoles may be contributing to the distribution of CBB damage vertically in coffee plants. Anoles are abundant in Puerto Rico and are able to exploit vertical niche space, including coffee bushes (Monagan et al. 2017; Perfecto and Vandermeer 2020). Anoles prey on CBB and are negatively associated with *W. auropunctata* (Monagan et al. 2017; Perfecto and Vandermeer 2020). *W. auropunctata* nests, whether associated with shade trees and coffee plants or not, are mainly on the ground, which mean their activity is likely limited to the lower portions of the coffee plants (Wetterer and Porter 2003). *W. auropunctata* predation of CBB corresponds with less CBB survival, as the ants are entering the berries in order to prey on CBB. This results in damage to the berry whether the CBB survives or not. Anoles may maintain activity in the top branches of coffee bushes, avoiding *W. auropunctata* and preying on free roaming CBB, thus resulting in less CBB damage in the upper branches.

Ant community

Despite literature pointing toward the efficacy of diverse ant communities in controlling CBB, different results were found here. In our study, species richness shows no impact on CBB damage or survival overall or in either farm individually. There were multiple species on each farm, but two species dominated, *W. auropunctata* in Finca Gran Batey and *S. invicta* in Finca Cítricos Inc. (Table 2). All the other ant species had relatively low abundance, which likely dampened the potential for species richness effects. Furthermore, given that the most common species in this study had opposite effects on CBB survival, it is possible that these effects cancel each other resulting in non-significant effects. A study in Colombia found that soil dwelling ants in shaded coffee removed significantly more CBB than in sun coffee where there was less ant diversity (Armbrecht and Gallego 2007). Similarly, other studies have suggested that a diverse ant community can significantly reduce CBB infestation (Gallego and Armbrecht

2005; Vélez Hoyos et al. 2006; Larsen and Philpott 2010; Morris et al. 2015). In addition, ant diversity and its effects on CBB is often examined in tandem with sun versus shade coffee (Armbrecht and Perfecto 2003; Gallego and Armbrecht 2005; Mariño et al. 2016), while this study look at ant community effects solely under shaded coffee farms. It remains unknown whether ant diversity effects on CBB have a saturating point or whether certain ant community assemblages outdo others in terms of CBB removal.

Variation between farms

Finca Gran Batey has significantly more CBB damage on average than Finca Cítricos, Inc. In addition, Finca Cítricos, Inc. has higher CBB survival than Finca Gran Batey. Finca Gran Batey is dominated by *W. auropunctata*, while Finca Cítricos, Inc. is dominated by *S. invicta*. The species composition of the farms may drive this pattern. *S invicta* is a bigger ant and cannot predate CBB embedded in coffee berries, so CBB faces less predation inside the berries in plants dominated by *S. invicta* than *W. auropunctata*, which can penetrate the berries and prey on the embedded CBB within coffee berries.

Wasmannia auropunctata

W. auropunctata is a Neotropical ant that has spread globally as a highly invasive species (Wetterer and Porter 2003). It spreads well in human disturbed areas, including agriculture (Armbrecht et al. 2001). In coffee farms it can cause yield declines because farm workers avoid areas dominated by this species due to its painful sting (Smith 1965) In this study, *W. auropunctata* decreased CBB damage in Finca Gran Batey and CBB survival overall, which aligns with past research on *W. auropunctata* and CBB (Jaramillo et al. 2006; Morris and Perfecto 2016; Morris et al. 2018). *W. auropunctata* has been shown to serve as a natural enemy to CBB both in terms of preventing damage and lowering survival of the CBB once it penetrates the berries. In Mexico, an ant exclusion experiment showed *W. auropunctata's* ability to significantly reduce CBB damage to coffee berries by preventing colonization of the berries by the CBB (Gonthier et al. 2013). In Cuba, Colombia, and Mexico, *W. auropunctata* reduced CBB survival by entering berries and preying on immature stages of CBB and by decreasing CBB colonization (Bustillo et al. 2002; Vázquez Moreno et al. 2006; Gonthier et al. 2013; Morris and Perfecto 2016). *W. auropunctata*'s small size, aggressive behavior, and ability to form super-colonies makes it easy for it to deter other ants species on coffee bushes and enter the coffee berries to prey on immature stages of CBB (Wetterer and Porter 2003; Le Breton et al. 2004; Errard et al. 2005)

Solenopsis invicta

S. invicta is native to South America and has become invasive in many regions (Adkins 1970). This species forms magnificent mounds and can bite and sting other wildlife as well as humans (Markin et al. 1975; Goddard 1996). In this study, *S. invicta* decreased CBB damage overall and increases CBB survival in Finca Gran Batey, where it was the most abundant species. *S. invicta* is a large and aggressive ant that prefers sunny areas of farms (Allen et al. 2004; Torres 1990). No studies have examined *S. invicta* as a natural enemy of CBB, however studies on *Solenopsis geminata*, an ant very similar to *S. invicta*, gives insight on how *S. invicta* and CBB may interact. One study found that *S. geminata* indirectly shields CBB from predation by other ants, and in its absence, there is significantly more predation of CBB (Trible and Carroll 2014). In contrast, *S. geminata* has also been found to prey on CBB adults and result in less CBB infestation (Vélez Hoyos et al. 2006; Vázquez Moreno et al. 2009). In this insistence in Puerto Rico, it is likely that *S. invicta* is preying on adult, free roaming CBB which results in less CBB damage on the coffee berries. Despite *S. invicta*'s predation of free roaming CBB, there is still an increase in CBB survival in its presence in Finca Gran Batey. The higher survival of CBB within the coffee berries can be explained by the inability of *S. invicta* to penetrate the berries dues to its larger size. Plants that are dominated by *S. invicta* very rarely have other species of ants, including *W. auropunctata*. A previous study in the same sites as this study, reported that out of 1320 samples, *W. auropunctata* was found on the same coffee plants as *S. invicta* in only 9.5% of the plants (Perfecto and Vandermeer *in review*). This suggests that in *S. invicta* dominated coffee plants, *W. auropunctata* will not be able to penetrate the damaged coffee berries and predate on the CBB (Yitbarek 2016). Our results suggest that *S. invicta* will only be effective at reducing CBB damage (i.e. pre-berry penetration), while *W. auropunctata* would be effective in reducing the CBB pre and post berry penetration and reduce CBB infestation and population density.

Monomorium floricola, Pheidole moerens, and Brachymyrmex heeri

M. floricola is a arboreal ant species from Asia and can be found across the tropics (Eow and Lee 2007; Wetterer 2009). It is small and slow moving, but it can sting and it tends honeydew insects (Macgown and Hill 2010). *P. moerens* is another small ant, native to the West Indies and primarily feeds on seeds but will forage on insects as well (Martinez et al. 2011). The presence of *M. floricola* and *P. moerens* increased CBB damage. These two species tend to have large colonies and to dominate those coffee plants where they are found. When *P. moerens* and *M. floricola* activity in coffee bushes was high (60% of baits occupied or more) *W. auropunctata* and *S. invicta* had little to no activity (Table 6 & 7). High densities of *M. floricola* and *P. moerens* in coffee bushes effectively shut out ants capable of predating CBB, leaving CBB without any control on these coffee bushes.

Table 6: Average Wasmannia auropunctata and Solenopsis invicta activity at varying levels of Monomorium floricola activity

Monomorium floricola (% baits occupied)	Wasmannia auropunctata activity (% baits occupied)	Solenopsis invicta (% baits occupied)
0%	54.9%	21.8%
20%	13.3%	15.6%
40%	20.0%	20.0%
60%	0%	0%
80%	-	-
100%	0%	0%

Table 7: Average Wasmannia auropunctata and Solenopsis invicta activity at varying levels of Pheidole moerens activity

Pheidole moerens (% baits occupied)	Wasmannia auropunctata activity (% baits occupied)	Solenopsis invicta (% baits occupied)
0	53.5%	22.5
20	24%	4
40	20%	0
60	6.7%	0
80	0%	0
100	0%	0

Brachymyrmex heeri is a common small ant distributed throughout The Caribbean, South America and Central America. In. spite of its wide distribution and abundance, there is very little known about this species. In this study, *B. heeri* had no effect on CBB damage or survival. Contrary to *P. moerens* and *M. floricola*, this species does not dominate individual coffee plants and they seem to have relatively small colonies. The non-significant effect of this species on the CBB is not surprising given that it is found on plants that have any of the other four species included in this study which show both positive and negative effects on the CBB.

Conclusion

Wasmannia auropunctata and Solenopsis invicta, in spite of being invasive species and considered pests because of their painful stings and tendency to form monospecific patches (Smith 1965; Adkins 1970; Wetterer and Porter 2003), can also be a key part of conservation biocontrol in Puerto Rican coffee farms. Both of these species reduce CBB damage, to some extent, but only W. auropunctata also reduces CBB survival, while S. invicta increases CBB survival. It is likely that the effect of *S. invicta* on the survival of the CBB is due this species' dominance of plants where they forage, and thus, S. invicta deters other smaller species, most likely *W. auropunctata*, away from coffee plants. Since *S. invicta* is a larger ant, it cannot penetrate the berries through the holes made by the CBB and cannot prey on CBB adults and brood inside the berries. Therefore, plants with *S. invicta* may suffer less attacks by the CBB, but those CBB that manage to overcome this initial protection, are more secure from predations by smaller ants and other predators that can penetrate through their galleries.

With high infestation rates and low implementation of pest control across the coffee region of Puerto Rico, conserving habitat on farms for natural enemies and reducing insecticide applications can serve as a path of least resistance towards pest control. However, these two species will need to be managed in a way that their activity on coffee plants is reduced during the harvest period, when workers are collecting berries and are exposed to their stings. A farmer in Cuba is experimenting with augmentation of nests of the big-headed ant, *Pheidole megacephala*, for the reduction of *W. auropunctata* activity during the harvest period. *P. megacephala,* appears to displace *W. auropunctata* temporarily, but enough to decrease W, auropunctata's activity on coffee plants at the time that people are completing the coffee harvest (Ivette Perfecto, personal communication with the farmer in Cuba). Furthermore, *W. auropunctata* and *S. invicta* appear to engage in strong competitive interactions in the farms 2`when they both are present (Perfecto and Vandermeer, in review). *S. invicta* has several phorid fly parasitoids that attack it (Feener and Brown 1992; Morrison and Porter 2005) which further complicates the interactions between these two ant species (Morrison 1999). These interactions need to be studied further in order to manage these species to enhance their biological control effects. Efforts to conserve natural enemy habitat such as reduced pruning, planting a polyculture of shade trees, and limiting any pesticide spraying may bolster not only W. auropunctata and S. invicta's ability to buffer against pest outbreaks, but a more wholistic ant community pest outbreak buffering as well. Further research should analyze the role of anoles in these coffee farms to examine how they fit into the larger picture of minimizing crop damage by CBB. The web of pests in the coffee-agroecosystem is complex but encouraging natural enemies may be the way to restore a balance in these insidious pest populations.

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