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Short communication

Testing the potential for ant predation of immature coffee berry borer (*Hypothenemus hampei*) life stages

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ABSTRACT

The coffee berry borer (CBB) is a devastating pest of coffee around the world. One potential control strategy is to conserve habitat for natural enemies of CBB in coffee agroecosystems so they can better suppress populations of this pest. Ants predate adult CBB and reduce infestation levels on coffee plants, but few experiments have tested their ability to consume immature CBB inside of fruits. This could prove an important part of controlling CBB populations since berries house many immature individuals on coffee plants during and between growing seasons. Here we experiment with *Wasmannia auropunctata* and *Solenopsis picea*, two species of ants that are commonly found nesting and foraging on coffee plants in southern Mexico. We performed a predation experiment in the laboratory, where ant nests of each species were exposed to CBB larvae and pupae extracted from fruits and removal rate was recorded over time. We also tested the ants' ability to enter CBB galleries in infested coffee fruit. We found that both ant species removed CBB immature individuals in the laboratory, but that *W. auropunctata* removed them significantly faster than *S. picea*. While both species showed increased activity after exposure to infested fruits, only *W. auropunctata* maintained activity around fruits and readily entered CBB galleries in fruits, despite documenting this behavior with *S. picea* outside of experimental trials. These results suggest that some ant species, which forage on coffee and are small enough to enter CBB galleries, have potential to contribute to biocontrol by consuming immature CBB inside infested fruits. However, further experiments in the field under realistic conditions will be needed to fully estimate this effect.

1. Introduction

Coffee is an agricultural crop with unique importance to human society. It is a major global commodity, serving as a source of livelihood for more than 20 million farming families around the world (Vega et al., 2009). It is also grown throughout the tropics in some of the world's most important biodiversity hotspots (Moguel and Toledo, 1999), and furthermore, it has been shown that the intensification of coffee farm management (i.e. use of chemical inputs, reduction of shade trees, removal of epiphytes, etc.) can impact the conservation of biodiversity in and around coffee farms (Jha et al., 2014; Perfecto et al., 1996; Philpott et al., 2008). However, shaded farms with low chemical inputs can provide habitat for a high level of biodiversity, including many species that are natural enemies of coffee pests (Jha et al., 2014; Philpott et al., 2008). This suggests that coffee farms may be ideal systems for the implementation of conservation or autonomous biocontrol (Perfecto and Vandermeer, 2015; Vandermeer et al., 2010) – where farmers benefit from pest control services provided by natural enemies by supporting their populations indirectly through the maintenance of habitat on farms (Barbosa, 1998). This strategy may be particularly effective in control of the coffee berry borer (*Hypothenemus hampei*, Ferrari, Coleoptera: Curculionidae), the worst insect pest of coffee around the world (Jaramillo et al., 2006).

The coffee berry borer (CBB) is a notorious coffee pest, causing annual loses of over $500 million in production globally (Vega et al., 2015). These beetles bore into coffee fruits, directly attacking the harvested crop, where they lay their eggs to complete their life cycles (Damon, 2000). Over the last decade evidence has accumulated that ants are important natural enemies of CBB on coffee farms. Some studies have demonstrated that ants will predate CBB in the laboratory (Armbrecht and Gallego, 2007; Larsen and Philpott, 2010; Philpott et al., 2012), while others have shown more direct evidence that ants can reduce CBB infestation on coffee plants in farms (Gonthier et al., 2013; Jiménez-Soto et al., 2013; Morris et al., 2015). Gonthier et al. (2013) showed that at least six different species of ants that forage on plants in Mexico can prevent free-roaming CBB adults from colonizing berries. Other studies have shown experimentally

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that smaller ant species can readily enter CBB holes in berries and remove adults from their galleries (Gallego Ropero and Armbrecht, 2005; Larsen and Philpott, 2010).

While ant predation of adult CBB contributes to the suppression of this pest, once CBB adults have oviposited, infested berries act as a source of many new CBB individuals; a single female CBB can lay over 100 eggs (Damon, 2000; Vega et al., 2015). Indeed, many berries that are missed during harvest remain on plants and become important refugia for CBB that supply the next season's population with individuals (Baker and Barrera, 1993; Gonthier et al., 2013). Additionally, the CBB lifecycle can take up to a month to complete, with the majority of time (more than 20 days) spent in the larval or pupal life stage (Damon, 2000). Because of these natural history attributes, predation of immature forms of the CBB inside fruits on coffee plants may be crucial for effective biological control. While some literature anecdotally suggests this occurs with ants (Bustillo et al., 2002; Fonseca and Araujo, 1939; Vázquez Moreno et al., 2006; Vélez et al., 2006), few studies report experimental evidence to support these claims. One study showed that several species of ants will predate CBB immature forms that have been removed from berries in the laboratory (Varón et al., 2004). However, the same study failed to show that these species significantly reduced CBB brood by removing them from parchment berries (dried seed with pulp removed) placed on the ground in farms. This may have been because some of the ant species tested, such as *Solenopsis geminata*, were too large to enter CBB galleries (Trible and Carroll, 2014; Varón et al., 2004). Another study reported observations of *S. geminata* removing immature CBB from parchment berries during parabolic solar drying, but similarly failed to find a significant removal effect of ground-foraging ants in experiments (Vélez et al., 2006).

Here we test the predation potential of ants on immature stages of coffee berry borer in the laboratory with two common ant species from a farm in southern Mexico, *Wasmannia auropunctata* and *Solenopsis picea*. We chose these species because they are common at our field site, they actively nest and forage on coffee plants (Gonthier et al., 2013; Perfecto and Vandermeer, 2013), and because other studies have reported their ability to predate adult forms of CBB (Armbrecht and Gallego, 2007; Gallego Ropero and Armbrecht, 2005; Gonthier et al., 2013). Additionally, these species appeared small enough to enter CBB galleries in berries, a necessary quality for CBB immature predation. Furthermore, their native ranges are widespread throughout Latin American (AntWeb, 2016), making them potentially important species for biocontrol throughout this coffee growing region.

2. Materials and methods

2.1. Study site and nest collection

All ant nests were collected from Finca Irlanda, a 280-ha, shaded, commercial polyculture coffee farm in the Soconusco region of Chiapas, Mexico. Ten *Wasmannia auropunctata* nests were collected in August, 2014 and ten *Solenopsis picea* nests were collected in July, 2015. Nests were collected by scraping moss, epiphytes, and bark from the base of coffee plants and shade trees, under which these species commonly nest at our site. Each individual nest was collected from different plants to increase the likelihood that the ants belonged to different colonies, however, in some cases ants may have belonged to the same clonal strain, as boundaries between colonies of these species are sometimes unclear (Perfecto and Vandermeer, 2013; Wetterer and Porter, 2003). The number of workers per nest was not counted, but nest containers were filled to approximately the same level with ants and nest material (vegetation and bark from nest collection). Nests were kept in the laboratory in one-liter, clear, plastic containers covered with perforated, clear lids with holes large enough for workers to pass through. Additionally, small vials with water-soaked cotton were placed inside nest containers. Ants were kept in the laboratory for no more than 72 h before performing trials, and thus were not fed artificial diets.

2.2. Immature predation

To test the potential for these species to predate immature coffee berry borers inside berries we performed two separate laboratory experiments. First, we exposed ant nests to five immature CBB individuals (a mix of larvae and pupae) removed from infested coffee berries collected from the field. Individuals were placed on metal platforms on top of ant nest containers so they could be easily observed. We monitored nests every 30 min to count the number of CBBs remaining on platforms for five hours total. We also did this with empty containers as controls to ensure that ants were responsible for CBB removal.

2.3. Fruit entry

For the second experiment we exposed ant nests to intact, but heavily infested CBB coffee fruits that were left on coffee plants during the previous harvest. On top of each nest container we placed one infested fruit with at least two CBB gallery holes. We monitored nests every 30 min over five hours to track the activity of ants, measured as presence or absence, either on the platform near fruits, on fruits, or visibly passing into CBB galleries in fruits. Finally, at the end of the five-hour period we dissected these berries to check for the presence of the ants inside. In addition to the experiments, we filmed (Video 1 and Video 2) and photographed (Fig. 2) ants entering CBB galleries in fruits to provide further evidence of this behavior.

Video 1.

Video 2.