Ants defend coffee from berry borer colonization

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Abstract Ants frequently prevent herbivores from damaging plants. In agroecosystems they may provide pest control services, although their contributions are not always appreciated. Here we compared the ability of eight ant species to prevent the coffee berry borer from colonizing coffee berries with a field exclusion experiment. We removed ants from one branch (exclusion) and left ants to forage on a second branch (control) before releasing 20 berry borers on each branch. After 24 h, six of eight species had significantly reduced the number of berries bored by the berry borer compared to exclusion treatment branches. While the number of berries per branch was a significant covariate explaining the number of berries bored, ant activity (that varied greatly among species) was not a significant factor in models. This study is the first field experiment to provide evidence that a diverse group of ant species limit the berry borer from colonizing coffee berries.

Keywords Biodiversity · Ecosystem services · Pest control · Ant–plant defense · Coffee · Agroecology · Ant · Formicidae

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Introduction

Ants benefit plants (Styrsky and Eubanks 2007; Chamberlain and Holland 2009; Rosumek et al. 2009; Trager et al. 2010). Humans have known this for quite a long time. In fact, ants were described as biological control agents in China around 304 AD (van Mele 2008). Many plants have also evolved to promote the activity of ants on their tissues. Surveys of tropical forests show that up to one third of all woody plants have evolved ant-attracting rewards (Schupp and Feener 1991). Some plants provide domatia as ant housing structures, while others attract ants to their tissues with extra-floral nectaries. Some plants are hosts to honeydew-producing hemipterans that excrete honeydew, a sugary substance consumed by ants. Still other plants are simply substrates for ant foraging. The majority of studies conducted across these ant–plant groups show that ants benefit plants by removal of

herbivores (Chamberlain and Holland 2009; Rosumek et al. 2009; Trager et al. 2010). Nonetheless, in many agroecosystems, the benefits of pest control services by ants are not recognized. Agricultural managers often view them as pests or annoyances to agricultural production because some ants tend honeydew-producing insects that can damage crops (Styrsky and Eubanks 2007). However, a review of the literature on ant-hemipteran associations suggests that even these associations benefit plants indirectly because ants remove other, more damaging herbivores (Styrsky and Eubanks 2007, 2010). Regardless, the literature lacks studies investigating ant–plant interactions in agroecosystems. Here, we broadly survey the pest control services provided by a suite of ant species to better understand the role of ant defense of coffee.

Coffee is a tropical crop that occurs as an understory shrub in its native range, and coffee plants are therefore often grown under a canopy of shade trees in agroforestry systems in some parts of the world (Perfecto et al. 1996). This canopy layer provides plantatsions with a forest-like vegetation structure that can help maintain biodiversity (Perfecto et al. 1996). Ant biodiversity is high in many coffee plantations and ants attack and prey on many coffee pests, including the coffee berry borer (CBB; *Hypothenemus hampei* [Ferrari] [Coleoptera: Scolytidae]) (Armbrecht and Gallego 2007; Philpott and Armbrecht 2006; Vandermeer et al. 2010). For example, *Azteca instabilis* F. Smith is a competitively dominant ant that aggressively patrols arboreal territories in high densities and previous research has found that it impacts the CBB (Larsen and Philpott 2010; Perfecto and Vandermeer 2006). Some laboratory and observational field studies have found that *Pseudomyrmex* spp., *Procryptocerus hylaeus* Kempf, and *Pheidole* spp. may limit the CBB (Jiménez-Pinto et al. Unpublished work; Philpott et al. 2012). However, other field experiments have not found ants to be biological control agents of the CBB (Varón et al. 2004; Vega et al. 2009). Further, the pest control effects of many ant species on the CBB have not yet been evaluated and it could be that previously documented effects are specific to only a few species.

Natural ant pest control of the CBB is particularly important because chemical insecticides used to control CBB are not always effective. This lack of effectiveness is in part because the CBB lifecycle takes place largely hidden within coffee berries (Vega et al. 2006) and also because the CBB has developed insecticide resistance (Brun et al. 1990). Several of the stages of the CBB life cycle make it vulnerable to attack by ants (Damon 2000; Jaramillo et al. 2007). First, the CBB hatches from eggs within the coffee berry, where it consumes the seeds (Damon 2000; Jaramillo et al. 2007). Small ants may enter the berry through the beetle entrance hole and predate the larvae and adults inside (Larsen and Philpott 2010; Perfecto and Vandermeer 2013). Second, old berries infested with the CBB may not be harvested because they often turn black and remain on the coffee branches or may fall to the ground (Damon 2000; Jaramillo et al. 2007). These old infested berries may act as a population reservoir of borer populations and ant predation at this stage could be very important for limiting CBB populations in the next season. Third, as adult borers disperse (flying or crawling) to colonize new berries, ants may prevent them from entering new berries (Pardee and Philpott 2011; Perfecto and Vandermeer 2006; Philpott et al. 2012). To date, no field experiment has specifically investigated how coffee-foraging ants limit CBB colonization of berries. Here, we studied the abilities of eight ant species to prevent colonization of berries by the CBB. We hypothesized that only species with high activity on branches would limit CBB colonization of berries. We show that six of eight ant species limit CBB colonization of berries and that the effect of ants is independent of ant activity on branches. This study is the first field experiment to provide evidence that a diverse group of ant species limits the CBB from colonizing coffee berries.

Materials and methods

Our research was conducted on Finca Irlanda, a coffee plantation in the Soconusco region of southern Mexico and the site of much ongoing research regarding community ecology of the arthropod interaction web (Vandermeer et al. 2010). In this region, the CBB is a major pest of coffee (Vandermeer et al. 2010). We searched for coffee bushes occupied by one of eight species that were each abundant enough to obtain sufficient replication for this experiment: *A. instabilis* ($N = 20$), *Crematogaster* spp. ($N = 20$), *Pheidole synanthropica* Longino ($N = 19$), *Pseudomyrmex simplex* Smith ($N = 30$), *Pseudomyrmex ejectus* Kempf ($N = 28$), *Solenopsis picea* Emery ($N = 31$),

Tapinoma sp. ($N = 30$), and *Wasmannia auropuncta-ta* Roger ($N = 28$) ($N = $ sample size used in experiment). Our goal was to capture a broad survey of the ant species that occupy the coffee vegetation in the coffee plantation. Within the plantation, five *Crematogaster* spp. forage in the coffee, however field identification at the time was not reliable therefore taxonomic resolution for *Crematogaster* spp. remained at the genus level. For *P. simplex* and *P. ejectus* it was not always possible to find occupied bushes by observation of ant foraging. Instead, for *P. simplex* and *P. ejectus*, we determined occupation by removing all dead twigs on the coffee bush and searching these for ant nests within the hollow branches (e.g. Philpott and Foster 2005). We reattached the nested hollow branch to a living branch with thin wire and treated these bushes as bushes occupied by *P. simplex* or *P. ejectus*.

To test the effects of each ant on CBB colonization of berries, we performed an ant exclusion experiment. We surveyed bushes occupied by one of the eight target ant species. We excluded coffee bushes with few branches to control for the size of the foraging area of each ant species. On each bush, we searched for two branches of equal age and position and roughly the same number of coffee berries (never more than eight berries difference). On each branch, we removed all berries that had CBB entrance holes. We then removed all ants from one branch and applied tanglefoot (exclusion) to the base of the branch near the coffee trunk. On the second branch, we left ants to forage freely (control). To estimate ant activity, we counted the total number of ants foraging on the stem, leaves, and berries of each branch for 1-min including those that travelled onto the branch during the 1-min survey. We also counted ants on exclusion branches after the experiment and if a branch had more than one ant individual present, we excluded the bush from analysis (this occurred in only two cases). To release CBB onto control and treatment branches, we created a leaf platform to aid their chances of encountering berries. The leaf platform consisted of a coffee leaf that we cut in two places on one side of the leaf. The leaf was wedged between the branch stem and a cluster of berries to create a platform surrounding the cluster (Online Resource 1). A coffee leaf was used as a platform because artificial structures attract attention from many ant species. After waiting several minutes to ensure normal ant activity, we released 20 CBBs on the leaf platforms of the control and exclusion branches. After 24 h, we counted the number of berries per branch that had CBBs inside entrance holes. We did not count partially bored holes in berries, nor CBBs that had bored into twigs and leaves. Multiple bored entrance holes per berry were only counted as one bored berry. We modified the experiment slightly for *P. simplex* and *P. ejectus* because of the difficulty in locating these species within a bush using visual cues (see above). For these two species, we used the living branch to which the nest was attached to as the control branch (with ants). This was done because we wanted to make sure that ants were actively foraging on control branches after the disturbance of removing nests.

To statistically analyze experimental data, we opted to use linear mixed models instead of paired t tests because mixed models allow inclusions of experimental non-independencies through the incorporation of covariates. We included bush as a random effect in the model to pair control and exclusion branches within each bush. Ant species (each of the eight ant species) and treatment (control or exclusion) and the species × treatment interaction were included as fixed effects in the model. To control for differences between each branch and bush, we included the number of berries per branch, the number of berries in contact with the leaf platform, and the number of worker ants per branch (ant activity) as covariates in the model. We performed type III F tests of significance for main effects with maximum likelihood (ML) to estimate the fixed effect parameters and variance of random effects (West et al. 2007). We removed non-significant factors from models and compared nested and null models with likelihood ratio tests to determine the best-fit model. We also compared ant activity (per minute) across different species to determine if this factor might correlate with berries bored and vary across ant species. To determine if ant activity correlated with the number of coffee berries bored, we limited the dataset to only control branches (with ants) and used a generalized linear model with a Poisson log-link function because data did not meet the assumptions of normality. To determine if ant activity varied by species, we again limited the dataset to only control branches and used ANOVA with Tukey's HSD analysis. We tested the normality of the data with qq-plots and Kolmogorov–Smirnov tests of model residuals. We conducted all statistical analyses with SPSS (20.0).

Fig. 1 Means (±SE) of the number of berries bored by CBBs (per branch) across ant species treatments and ant-exclusion treatments. Asterisks indicate significant differences between control (ant) and exclusion (no ant) treatments
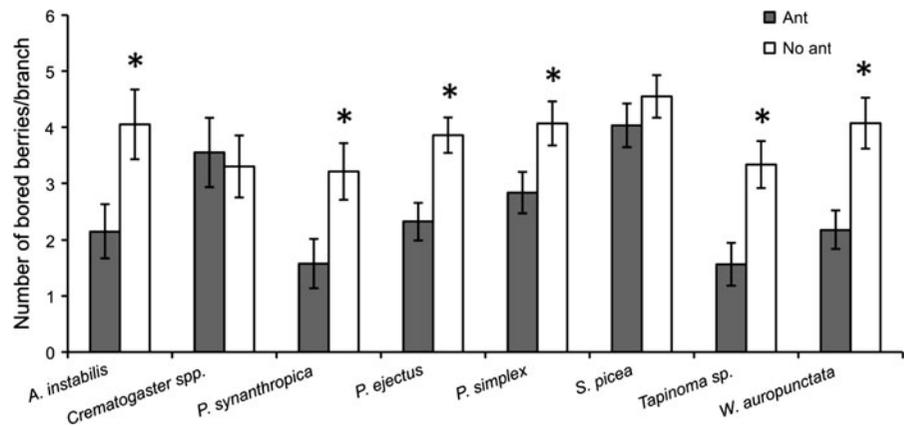
Results

The linear mixed model showed that the number of berries bored varied by ant species ($F_{7,206} = 3.5$, $P = 0.0013$), exclusion treatment ($F_{1,208} = 44.9$, $P = 0.0001$), and by the number of berries per branch ($F_{1,210} = 7.8$, $P = 0.0058$). There was no interaction between ant species and exclusion treatment ($F_{7,206} = 1.8$, $P = 0.0961$). Overall, pooling all ant species together, there were 50 % more berries bored in exclusion branches relative to controls (Fig. 1). Pair-wise comparison of control (ant) and exclusion (no ant) branches revealed that six of eight ant species significantly reduced the number of berries bored relative to controls. On *A. instabilis* control branches, there were 88 % fewer CBB in berries, with *P. synanthropica* there were 200 % fewer, with *P. ejectus* there were 66 % fewer, with *P. simplex* there were 43 % fewer, with *Tapinoma* sp. there were 210 % fewer, and with *W. auropunctata* there were 86 % fewer bored berries relative to their paired exclusion branches (Fig. 1). There was no difference between the number of bored berries on control and exclusion branches on bushes with *Crematogaster* spp. and *S. picea*. The number of berries in contact with the leaf platform and the number of ants per branch had no correlation with the number of berries bored.

Ant activity (ants per branch per minute) did not correlate with the number of berries bored (Wald $\chi = 1.6$, df = 1, $P = 0.204$), but did differ across species when only control branches were considered ($F_{7,206} = 25.6$, $P \leq 0.0001$). Across species, *Tapinoma* sp. and *W. auropunctata* had the highest activity, *A. instabilis*, *Crematogaster* spp., *P. synanthropica*, and *S. picea* had intermediate activity, and *P. ejectus*

and *P. simplex* had the lowest activity (as determined by Tukey's HSD; Fig. 2).

Discussion

Our study represents one of the first field experiments showing that a broad survey of ants reduce colonization of coffee berries by the CBB. This is in contrast to previous studies that suggest ants may not have any effects on CBB, especially in field experiments (Varón et al. 2004; Vega et al. 2009). Our results are in accordance with other observational studies that show that specific ant species may limit CBB in coffee plantations, yet these studies have either focused on the most dominant or abundant species observed (Jiménez-Pinto et al. Unpublished work; Perfecto and Vandermeer 2006; Perfecto and Vandermeer 2013) or investigated the broad community-wide impacts of ants on the CBB (Larsen and Philpott 2010). Our experimental approach is limited to our understanding of how ants control CBB colonization of berries and not other life stages of the CBB. Our study suggests that ant occupation of coffee bushes is very important during a seasonal period when new coffee berries develop and the CBB begins to disperse from old infested berries to developing un-infested berries (Damon 2000).

It is surprising that *Crematogaster* spp. and *S. picea* did not limit the colonization of berries, considering that other studies have shown species within these two genera have important effects on herbivores (Kaplan and Eubanks 2005; Philpott et al. 2008). Low ant activity on coffee bushes with *Crematogaster* spp. or *S. picea* cannot explain these results because these

Fig. 2 Means (±SE) of ant activity per branch per minute across the control branches of the ant species treatments. Common letters indicate means that are not significantly different from one another as determined by Tukey's HSD
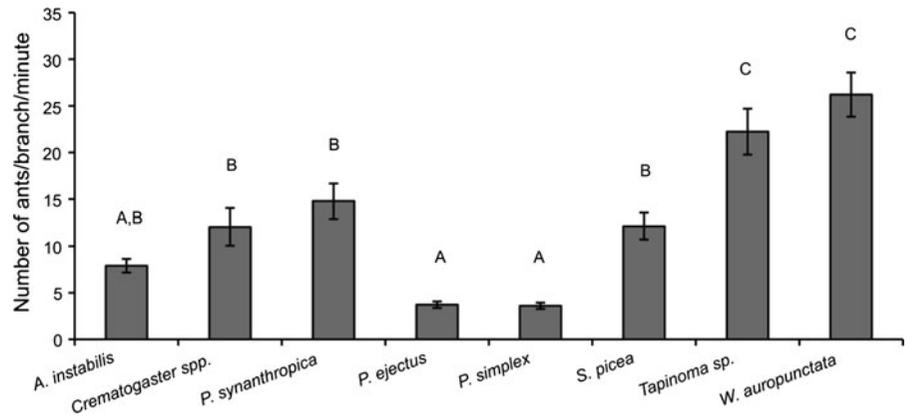
species had greater activity per branch than *P. ejectus* and *P. simplex* and equivalent activity to *A. instabilis* and *P. synanthropica*, species that did limit CBB damage. One explanation could be that because we grouped five *Crematogaster* spp. together into a single treatment, effects of individual species may be masked. *Solenopsis picea* may have an effect on CBB colonization, but only with higher ant activity or when CBB are in closer proximity to nest entrances. This species also has a small body size and moves relatively slowly in comparison to the species that did have an effect, which might have limited it from removing or easily capturing CBBs. *Wasmannia auropunctata* is of similar size to *S. picea* and still had strong effects on CBB. However, *W. auropunctata* had significantly higher ant activity on branches as compared to *S. picea*. Perhaps the combination of low activity, small body size, and slower movement limited *S. picea* from affecting the CBB. While we found no effect of *S. picea* on CBB colonization of berries, it may be that *S. picea*, and other smaller ants, have important impacts on the CBB at other stages of the CBB life cycle because they can pass into entrance holes of the CBB (Perfecto and Vandermeer 2013).

Experiments with both *P. simplex* and *P. ejectus* employed slightly different methodologies than the other ant species, which may have intensified the effect of these ants. For these two species, hollow twigs that contained ants were attached to a branch with berries and this branch was used as the control branch in the experiment. This likely elevated the number of ants per branch per minute. However, in the lab, *P. simplex* had similar effects on the CBB (Philpott et al. 2012). Additionally these two species

had the lowest densities on control branches of all other species, averaging 3.6 and 3.7 ants per branch for *P. ejectus* and *P. simplex,* respectively. Thus, these species have effects at very low numbers, and the results of this study should only pertain to branches for which the density of these species reaches this mark.

Certain aggressive ants (that spatially defend arboreal territories) that limit CBB colonization of berries might also benefit CBB after colonization. Larger ants cannot enter berries, but if they are aggressive competitors for space, they will prevent other ants from occupying the branches they patrol (Perfecto and Vandermeer 2013). These ants, likely *A. instabilis* and *P. synanthropica*, may provide CBB with enemy free space after the CBBs colonize berries in their territories.

In conclusion, we find that six of eight ant species limited CBB colonization of coffee berries suggesting that ants, generally, provide important pest control services within coffee agroecosystems. This is the first field experiment to demonstrate general ant limitation of CBB colonization. This finding is important considering that chemical pesticides are thought to be ineffective at controlling the CBB (Vega et al. 2006). Nonetheless, ants do not completely control the CBB, other control agents like birds, parasitoids, and fungal pathogens also aid in the control of the CBB (Vega et al. 2009). Further work should look at larger scale impacts of ants on the CBB, such as farm scale impacts. Also, more theoretical work is needed to understand how ants impact the CBB at different stages of its life cycle and to reveal which stage of the life cycle is most important for population regulation. Nonetheless, this study provides strong evidence that ants defend coffee from CBB colonization.

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