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High intermediary mutualist density provides consistent biological control in a tripartite mutualism

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ABSTRACT

Understanding the ecology of mutualisms becomes a particularly important task when considering agroecosystems, as many ecosystem services are associated with mutualistic interactions. Here we report on experiments associated with an indirect pest control mutualism between the arboreal nesting ant *Azteca sericeasur* and coffee. This system is particularly interesting because the indirect *Azteca-Coffea* mutualism emerges from an *Azteca*-scale insect mutualism that takes place on the coffee plant. We describe this interaction structure as a mutualism-dependent mutualism and ask whether the density of intermediary mutualist (scale insects, *Coccus*) that benefits *Azteca* also influences the benefits provided to coffee plants. We found that indeed *Azteca*'s benefit to *Coffea* is consistent when *Coccus* density is high. Furthermore, we also found that at low *Coccus* density *Azteca* only benefits *Coffea* in the beginning of the rainy season, and this effect is likely due to the fact that *Coccus* produces less sugars with higher precipitation. We suggest a framework for thinking about context-dependency in agroecosystem mutualisms that may provide a more mechanistic way to tease apart the prevalent context-dependent results in ecological literature. Finally, we address some past recommendations as it pertains to the management of the *Azteca-Coffea-Coccus* complex in coffee agroecosystems.

1. Introduction

The importance of mutualistic interactions in determining the structure and the function of natural ecosystems is well known (Boucher et al., 1982; Bronstein, 1994; Jha et al., 2012). Recently attention has been given to the value of these interactions in agroecosystems, as illustrated by a wide array of examples of ecosystem services associated with mutualisms such as pollination (Kremen et al., 2007; Lonsdorf et al., 2009; Morandin and Winston, 2006), seed dispersal (Boucher, 1982; Willson, 1993; Gammans et al., 2005), protection against herbivores (Perfecto and Vandermeer, 2006; Vandermeer et al., 2010; Pringle et al., 2011; Gonthier et al., 2013; Lanan and Bronstein, 2013; Dáttilo et al., 2015; De la Mora et al., 2015), and soil aeration (Lavelle et al., 1995). Many of these mutualistic interactions are frequently conceptualized as taking place in solely a pairwise fashion, but in reality almost of these interactions take place in the presence of other organisms and sometimes even depend on other organisms.

The three-organism mutualistic association that is fairly ubiquitous, in both natural systems (Boutcher, 1985; Bronstein, 1994) and in agroecosystems (Compton and Robertson, 1988; Liere and Perfecto, 2008) is the relation between ants and honeydew secreting hemipterans (Way, 1963; Perfecto and Vandermeer, 2006; Pringle et al., 2011; Styrsky and Eubanks, 2007). In coffee agroecosystems, the green coffee scale (*Coccus viridis*) has a pest status, it can reach high densities but only when it is in a mutualistic relationship with ants. Some authors have described that the green coffee scale can affect the coffee bean weight and the reducing the coffee photosynthesis rate by producing sooty mold on the leaves.

A tripartite association that is ubiquitous, both in natural systems (Boutcher, 1985; Bronstein, 1994) and agroecosystems (Compton and Robertson, 1988; Liere and Perfecto, 2008), is the relationship between ants, honeydew secreting hemipterans, and plants (Way, 1963; Perfecto and Vandermeer, 2006; Pringle et al., 2011; Styrsky and Eubanks, 2007). The outcomes of these associations can be variable through time and space and can have impacts on the plant that range from beneficial,

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harmful, or no net positive or negative effect (Buckley, 1987; Bronstein, 1994; Vandermeer et al., 2010; Jiménez-Soto et al., 2013; Morris et al., 2015). A well-studied example of an ant-hempiteran mutualism is the relationship between the carton nesting arboreal ant, *Azteca sericeasur* (Hymenoptera: Formicidae), and the green coffee scale, *Coccus viridis* (Hemiptera: Coccidae), which occurs in coffee agroecosystems (Perfecto and Vandermeer, 2006; Liere and Perfecto, 2008; Liere, 2011). *C. viridis* offers sugar resources to supplement the energetic needs of the *A. sericeasur* colony, and in return *A. sericeasur* protects *C. viridis* from natural enemies such as the coccinellid *Azya orbigera*, which is an important scale predator in the coffee agroecosystems (Liere, 2011; Jha et al., 2012). The *A. sericeasur*-*C. viridis* mutualism serves a potentially important function within coffee agroecosystems, as *A. sericeasur* attacks not only natural enemies of *C. viridis*, but also herbivores of coffee like the economically important coffee berry borer (CBB) (Gonthier et al., 2013; Perfecto et al., 2014; Morris et al., 2015). This results in *A. sericeasur* indirectly benefiting coffee while coffee provides a host for its mutualist (*C. viridis*), thus resulting in an indirect mutualism between *A. sericeasur* and coffee.

This coffee system offers a unique opportunity to study a complex arrangement of species interactions that is seen in other systems as well (Styrsky and Eubanks, 2007; Zhang et al., 2012). We essentially have a structure that can be described as a mutualism-dependent mutualism. Where the *Azteca*-*Coccus* system can be considered the primary mutualism, due to the fact that the *Azteca*-*Coffea* indirect mutualism only emerges from the *Azteca*-*Coccus* association, thus it is contingent on its presence. Although similar interaction structures are likely ubiquitous in ecosystems they have received very little attention, with most attention directed towards pairwise mutualisms (Heil, 2007). Furthermore, this system is particularly unique, as most other ant-plant studied could be considered ‘more specialized’ mutualisms, with plants that have domatia for ants (Pringle et al., 2011), directly offer nutrients to ants (Janzen, 1966), or both (Palmer and Brody, 2007; Palmer et al., 2010). Our system in coffee unique in that the interaction structure we are studying is comprised of organisms which are all newly sympatric. The *Coffea* genus was introduced to the Americas from Africa in the late 1700s (Pendergrast, 2010), *C. viridis* is thought to have been introduced from Brazil where it is native (Murakami, 1984), and *Azteca* is native to the region.

Here we ask if the dynamics of more complex interaction structures, such as the above mentioned mutualist-dependent mutualism, behave qualitatively similar to more thoroughly studied pairwise mutualisms. Density dependence has been a focus of many studies about mutualisms, and it has been exhibited in various systems (Breton and Addicott, 1992). In ants the link between increased sugar intake from mutualists and their ability to protect host plant or associated mutualist has been established (Pringle et al., 2011). In our study system a similar results has been described by Perfecto and Vandermeer (2006) when they found through surveys that CBB damage decreases on coffee plants with increasing densities of *C. viridis*. In this article we experimentally test the hypothesis that increasing *C. viridis* density (the intermediary mutualist) reduces the number of CBB damaged fruits, and thus increases *A. sericeasur*'s protection of coffee (Table 1).

2. Materials and methods

Our research was conducted on Finca Irlanda, a coffee agroecosystem, in the Soconusco Region of southern México (15°10'N y 92°20' W; 1090 msnm). The natural vegetation is typical of high forest and mid evergreen forest (Miranda and Xolocotzi, 1963). Finca Irlanda can be described as a shaded coffee agroecosystem which covers 300 ha, and contains a diverse community of about 200 species of shade tree with the genus *Inga* being dominant in the system (Vandermeer et al., 2002).

Table 1

Output from generalized linear mixed models for the number of fruits attacked by the CBB in 2014.

Fruits damaged by CBB	Estimate	z value	p-value
<i>2014 Control Low Reference</i>			
(Intercept)	1.012 ± 0.190	5.319	< .0001
Azteca activity	0.009 ± 0.011	0.862	.389
Control (High scale density)	−0.283 ± 0.225	−1.255	.21
Control (Medium scale density)	0.086 ± 0.207	0.417	.677
Exclusion (Low scale density)	0.275 ± 0.202	1.362	.173
Exclusion (High scale density)	0.379 ± 0.288	1.32	.187
Exclusion (Medium scale density)	−0.175 ± 0.279	−0.626	.531
<i>2014 Control Medium Reference</i>			
(Intercept)	1.098 ± 0.194	5.647	< .0001
Azteca activity	0.009 ± 0.011	0.862	.3886
Control (Low scale density)	−0.086 ± 0.207	−0.417	.677
Control (High scale density)	−0.369 ± 0.225	0.1008	−1.641
Exclusion (Medium scale density)	0.100 ± 0.211	0.475	.6346
Exclusion (Low scale density)	0.175 ± 0.279	0.627	.5309
Exclusion (High scale density)	0.555 ± 0.290	1.913	.0557
<i>2014 Control High Reference</i>			
(Intercept)	0.729 ± 0.227	3.213	.00131
Azteca activity	0.009 ± 0.011	0.862	.3886
Control (Low scale density)	0.283 ± 0.225	1.255	.20962
Control (Medium scale density)	0.369 ± 0.225	1.641	.10081
Exclusion (High scale density)	0.655 ± 0.23	2.803	.00506
Exclusion (Low scale density)	−0.379 ± 0.287	−1.32	.18667
Exclusion (Medium scale density)	−0.554 ± 0.289	−1.913	.05574

2.1. Field experiments

In order to understand how variable the outcomes of the *Azteca*-*Coccus*-*Coffea* complex is, we collected data across two years, where experiments were conducted in late August through early September of 2014, and in June of 2015. In a 45 ha plot we located plants with both *C. viridis* and *A. sericeasur*. In each year we found plants and chose six branches with varying densities of *C. viridis*. In 2014 the treatments are described as; Low (N = 15, minimum = 6, maximum = 28, median = 15, average = 15.47), Medium (N = 15, minimum = 20, maximum = 40, median = 27, average = 29.10), and High (N = 15, minimum = 26, maximum = 141, median = 60, average = 63.80), and it 2015; Low (N = 15, minimum = 6, maximum = 18, median = 14.5, average = 13.17), Medium (N = 7, minimum = 20, maximum = 38, median = 30, average = 30.14), and High (N = 15, minimum = 36, maximum = 120, median = 70, average = 67.45). On each plant, two branches were located and/or manipulated to fit into the low, medium, and high categories of *C. viridis* densities. One of the branches in each pair excluded *A. sericeasur* by putting the sticky substance tanglefoot in the base of the branch. On the branches that were not excluded, we measured ant activity by looking at the number of ants that pass a point on the branch for three minutes. Prior to the addition of CBB, we removed all the coffee fruits with CBB damage on both treatments. After 24 h we returned and placed 20 adult CBBs on each branch using a leaf platform as described by Gonthier et al. (2013) and Morris et al. (2015). All CBB were collected from fruit on the same farm. The number of coffee fruits with CBB damage was recorded 24 h after the release the CBBs.

2.2. Statistical analysis

We used generalized linear mixed models (GLMM) with a log link function and Poisson distribution to understand the effect of ants across *C. viridis* densities. To look at what factors are associated with the number of CBB damaged fruits, models included *A. sericeasur* activity as a covariate, the presence or absence of *A. sericeasur* as a categorical variable, *C. viridis* density as a categorical variable (high, medium, and low), and the interaction between *A. sericeasur* presence and *C. viridis*

density. We also used GLMMs to look at differences in *A. sericeus* activity across *C. viridis* densities. Again, this model included *C. viridis* densities as a categorical variable in the models, and used a Poisson distribution. Since each plant had multiple scale density treatments, we included a random effect in the GLMMs to account for potential similarities in within a plant/ant nest. Each year we analyzed 2014 and 2015 separately.

3. Results

A. sericeus activity significantly varied with scale insect density. We found that *A. sericeus* activity was significantly higher when compared with the medium (Estimate = -0.359 ± 0.201 , $z = -1.785$, $p\text{-value} = .0742$) and low density treatments (Estimate = -0.559 ± 0.122 , $z = -4.575$, $p\text{-value} < .0001$) but this was true only in 2014. In 2015 the high scale density treatment had significantly higher activity than the low scale density treatment (Estimate = -0.559 ± 0.122) but not the medium scale density treatment (Table 3). The key take away is a high *C. viridis* densities typically results in more *A. sericeus* activity.

In both 2014 and 2015 we found that there was a significant negative effect of *A. sericeus* ant exclusion on the number of fruits attacked by the CBB in the high *C. viridis* treatment (Fig. 1a, b, Table 2). In 2015 we also found a significant negative effect of ant exclusion in the low *C. viridis* treatment (Fig. 1b, Table 2). Furthermore, there was significantly less CBB damage on high *C. viridis* treatments than on medium *C. viridis* treatments (Estimate = -0.587 ± 0.269 , $z = -2.18$, $p\text{-value} = .0293$).

In the high *C. viridis* treatment there does not seem to be a difference in the size of the effect from ant exclusion between 2014 (Estimate = 0.655 ± 0.230) and 2015 (Estimate = 0.736 ± 0.238). Additionally, in 2015 when we saw an effect of ant exclusion on the low *C. viridis* treatment (Estimate = 0.476 ± 0.187), the estimated plus confidence intervals slightly overlaps with the high *C. viridis* treatment estimated effect minus its confidence intervals resulting in an overlap of 0.165. This result is suggestive that the effect of *A. sericeus* may not be greater with higher *C. viridis* density, which is in contrast to 2014's findings.

Fig. 1. Plot shows damaged fruits by the CBB for experimental treatments in 2014. Error bars are 95% confidence intervals and asterisk shows significant differences between *A. sericeus* & no *A. sericeus* treatments.

Table 2

Shows the output from generalized linear mixed models for the number of fruits attacked by the CBB in 2015.

Fruits damaged by CBB	Estimate	z value	p-value
2015 Control Low Reference			
(Intercept)	1.14 ± 0.188	6.052	< .0001
Azteca activity	0.01 ± 0.009	1.093	.2744
Control (Medium scale density)	0.291 ± 0.242	1.202	.2295
Control (High scale density)	-0.296 ± 0.216	-1.368	.1712
Exclusion (Low scale density)	0.476 ± 0.187	2.541	.0111
Exclusion (Medium scale density)	-0.264 ± 0.309	-0.853	.3939
Exclusion (High scale density)	0.259 ± 0.267	0.97	.3322
2015 Control Medium Reference			
(Intercept)	1.431 ± 0.232	6.154	< .0001
Azteca activity	0.01 ± 0.009	1.093	.2744
Control (High scale density)	-0.587 ± 0.269	-2.18	.0293
Control (Low scale density)	-0.291 ± 0.242	-1.202	.2295
Exclusion (Medium scale density)	0.212 ± 0.259	0.822	.4113
Exclusion (High scale density)	0.523 ± 0.333	1.569	.1168
Exclusion (Low scale density)	0.264 ± 0.309	0.853	.3939
2015 Control High Reference			
(Intercept)	0.844 ± 0.237	3.552	.000382
Azteca activity	0.01 ± 0.009	1.093	.274355
Control (Medium scale density)	0.588 ± 0.269	2.18	.02927
Control (Low scale density)	0.296 ± 0.216	1.368	.171249
Exclusion (High scale density)	0.736 ± 0.238	3.09	.001999
Exclusion (Medium scale density)	-0.523 ± 0.333	-1.569	.116757
Exclusion (Low scale density)	-0.259 ± 0.267	-0.969	.332308

Table 3

Shows the generalized linear mixed model outputs for factors influencing *A. sericeus* activity.

Azteca activity	Estimate	z value	p-value
2014 Medium Scale Density Reference			
(Intercept)	-0.195 ± 0.613	-0.318	.7502
High scale density	0.301 ± 0.141	2.127	.0334
Low scale density	-0.074 ± 0.154	-0.482	.6302
2014 High Scale Density Reference			
(Intercept)	0.106 ± 0.611	0.173	.86266
Medium scale density	-0.301 ± 0.141	-2.127	.03339
Low scale density	-0.375 ± 0.144	-2.598	.00938
2015 Medium Scale Density Reference			
(Intercept)	1.243 ± 0.238	5.216	< .0001
Low scale density	-0.2 ± 0.21	-0.954	.3402
High scale density	0.359 ± 0.201	1.785	.0742
2015 High Scale Density Reference			
(Intercept)	1.602 ± 0.181	8.838	< .0001
Medium scale density	-0.359 ± 0.201	-1.785	.0742
Low scale density	-0.559 ± 0.122	-4.575	< .0001

4. Discussion

Our experiments partially support the results of Perfecto and Vandermeer's (2006) observational study, but we show that the effects of *C. viridis* density on CBB biological control are more nuanced than previously thought. With high *C. viridis* density treatment consistently showing an effect from ants across both years, we suggest that this experiment provides what we understand as the first evidence of the reward of one mutualism to be dependent on the density of an intermediary mutualist. These results are quite clear in 2014, where *A. sericeus* reduced CBB infestation only in the high *C. viridis* treatment. Although we see the same effect in 2015 in the high *C. viridis* treatment, we see an effect that is qualitatively smaller but likely comparable in the low *C. viridis* density treatment. This suggests that the effect of *A. sericeus* in reducing CBB infestation in 2015 does not directly scale with *C. viridis* density, especially since we do not see any intermediate effect in 2015 from *A. sericeus* in the medium *C. viridis* density treatment. Interestingly, when looking at both years (Figs. 1 and 2) we

Fig. 2. Plot shows damaged fruits by the CBB for experimental treatments in 2015. Error bars are 95% confidence intervals and asterisk shows significant differences between *A. sericeus* & no *A. sericeus* treatments.

consistently see the effects between exclusion and control are higher in 2015 suggesting that something may be fundamentally different between the dynamics of the *Azteca-Coccus-Coffea* complex.

We propose that the observed differences between years may be due in part to the seasonal weather patterns in the time of the year the experiments were conducted. Where in 2014 the experiments were conducted in the height of the rainy season and in 2015 during the onset of the rainy season. This means that experiments were conducted at a wetter time of the year in 2014, and a drier time of the year in 2015. Ant mutualists rewards vary across time and space with precipitation patterns (González-Teuber et al., 2012, Pringle et al., 2013). In particular, extrafloral nectaries produce more rewards in the dry season (González-Teuber et al., 2012), and scale insects produce higher quantities of sugar in their honeydew during drier parts of the year (Pringle et al., 2013). Pringle et al. (2011) also was able to show that ants fed higher sugar diets did not necessarily have higher activity but did attack herbivores at higher rates. If *A. sericeus* is sugar limited we expect that in wetter times of the year (2014) when scales are producing less sugar, they focus on larger aggregations of *C. viridis* to acquire sugar and thus provide protection only in high *C. viridis* densities. The effect of *A. sericeus* in both high *C. viridis* and low *C. viridis* density treatments in 2015 may be a result of production of more sugar rich honeydew when it is drier. This suggests that ants are likely not sugar limited at the beginning of the rainy season and offer protection to the plant even at low *C. viridis* densities.

4.1. Defining useful ecological contexts

The role of seasonality on the outcome of the *Azteca-Coccus-Coffea* complex is not so surprising, as the role of conditionality or context dependency in the outcomes of ecological interactions has become a prominent feature of ecology (Bronstein, 1994; Chamberlain and Holland, 2009) and prevalent in mutualisms (Breton and Addicott, 1992; Bronstein, 1994). It seems as if the challenge of unpacking context dependency in ecology becomes to find a useful definition of context that may provide some mechanistic ways to understand the underlying ecology at play. This is where agroecosystems may provide unique insight into useful ways to conceptualize ecological contexts.

Because agroecosystems are always managed in some ways, different management types may be seen as offering different contexts in which the ecology in the system plays out. To illustrate this point, we can use the arbitrary example of pest suppression in coffee agroecosystems. A common practice in coffee agroecosystems is reduction of shade trees (Perfecto et al., 1996), and as a result this will reduce the nesting space (carrying capacity) of *Azteca* and at the extreme lead to a total breakdown in *Azteca-Coffea* indirect mutualism. Furthermore, we know that quality honeydew from hemipterans depends on nitrogen content in plants (Bi et al., 2001), thus the application of fertilizers may increase nitrogen quantity in plants in which *C. viridis* feed thus making a higher quality reward and a strengthening the mutualism between *Azteca-C. viridis*. Research on agroecosystem management with the purpose of creating contexts in which ecosystem services are encouraged is a natural extension of this concept. Work has already been done to show that management can indeed provide contexts under which ecological interactions change in ways that may be of interest to people who live, work, and depend on these agroecosystems (Morandin and Kremen, 2013; Cranmer et al., 2012).

4.2. Implications for the agroecosystem

It is widely acknowledged that a wide range of multi-trophic interactions play out within agroecosystems, and considering this complexity of interactions is essential for understanding implications of management practices (Vandermeer et al., 2010; Kessler and Heil, 2011; Koptur et al., 2015). If we acknowledge that different management practices provide different contexts for the interactions to play out in agroecosystems, then we can begin to think about approaching a predictive framework for understanding the details of how context (management) X leads to interaction (or modification of interaction) Y. Although many attempts have been made to do exactly this, they often fall short mainly due to an insufficiently accurate conception of how interactions are organized in a system. A good example is the recommendation that ants be eliminated from coffee agroecosystems (Hanks and Sadof, 1990; Kawat, 2010) mainly due to the fact that *C. viridis* has been described as a pest before. This suggestion likely originated from a conception of the coffee agroecosystem being organized as represented in Fig. 3B where the ant has an indirect negative effect on *Coffea*, and a positive effect on *C. viridis*. by protecting them from their natural enemies (here *Azya orbiger*). If this was an accurate representation of the community then certainly the removal of ants may very well have a net positive impact on *Coffea* (Ant removal is represented by moving from Fig. 3B to D). It is important to note that Fig. 3B will likely never exists in coffee agroecosystems as the CBB is one of the most widely distributed pests of *Coffea* around the world (Vega et al., 2015), and in addition to being widely distributed it is also far more damaging to production than *C. viridis*. Thus in practice the removal of ants from coffee agroecosystems would likely look something like moving from Fig. 3A to C, where the more severe pest of coffee (CBB) is released from the negative interaction of the ant, and *Coffea* is now attacked by both herbivores. We propose a reconsideration of such recommendations, and argue that a preservation of interactions within these agroecosystems will likely promote more effective control of major pests of coffee.

We suggest that in order to make any sensible recommendations on how to manage an agroecosystem one first must have an in depth understanding of the ecology at play in the system. This means making informed predictions on the potential effect the removal or addition of a particular organism will have on the system as a whole, but the whistle has been blown on the exceeding importance and impracticality of predicting the these kinds of effects (Simberloff and Stiling, 1996). We propose that instead of aiming to remove or add organisms to agroecosystems we should consider preserving organisms and the interactions among them until such an understanding of the ecology at play has been achieved. Part of this better understanding of the ecology of

Fig. 3. Conceptual interaction diagrams the following numbers correspond to the following organisms in the study system. 1. *Azteca sericeasur*, 2. *Coccus viridis*, 3. *Coffea*, 4. *Hypothenemus hampei* (CBB), 5. *Azya orbiger*. Panels A and B show two conceptualizations of coffee agroecosystem. Moving from A to C and from B to D show how the removal of ants from the system will alter interaction structures within each conceptualization. Solid arrows represent direct interactions, dotted arrow represent indirect interactions. Note the removal of *A. sericeasur* in conception A-C will result in net negative effect on *Coffea* due to presence of CBB, while removal of *A. sericeasur* in conception B-D will result in net negative effect on *Coffea*.

agroecosystems must include longer-term studies which encompass seasonal variation in the systems. Our study suggests someone studying the dynamics of the *Azteca-Coccus-Coffea* interactions in the dry season may come to very different conclusions than someone studying it in the rainy season, and management suggestions that may arise from either year individually could have unseen consequences for producers. We want to point out that research is emerging that suggests preserving interactions in these agroecosystems may offer a viable strategy for reducing the effects of the most impactful issues facing producers (Cruz-Rodríguez et al., 2016, Hajian-Forooshani and Rivera Salinas, 2016, Vandermeer et al., 2010, Koptur et al., 2015).

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