Investigating The Effects Of Ant-Hemipteran Mutualisms On The Invertebrate Community Structure And Their Host Plant, Honey Mesquite (prosopis Glandulosa)

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INVESTIGATING THE EFFECTS OF ANT-HEMIPTERAN MUTUALISMS ON THE INVERTEBRATE COMMUNITY STRUCTURE AND THEIR HOST PLANT, HONEY MESQUITE (*PROSOPIS GLANDULOSA*)

A Dissertation Presented

by

Nabil Nasseri

to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy Specializing in Biology

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ABSTRACT

Ants are ubiquitous in most communities and many form opportunistic mutualisms with honeydew-producing hemipterans (e.g. treehoppers). Hemipterans excrete honeydew, a carbohydrate rich substance, that ants harvest and, in return, ants protect their honeydew-producing partners from parasitoids, predators, and competitors. Given the efficacy of tending ants in removing hemipteran antagonists, and the strong roles that ants play within their communities as predators, competitors, and seed dispersers, surprisingly little is known of the effects of ant-hemipteran mutualisms (AHM) on the invertebrate communities in which they are embedded or on the plants that host AHM. Using observational and manipulative field experiments, I examined the long-term effect of AHM on their host plant’s, honey mesquite (Prosopis glandulosa), reproductive potential and quality. In addition, I measured how the presence of AHM affects the abundance, richness, diversity, and composition of the invertebrate communities living on honey mesquite.

Plants hosting AHM may indirectly benefit (through the removal of herbivore arthropods) or suffer (through the loss pollinators) due to the defensive behavior of tending ants. To determine the effects of AHM on their host plant, I established a four-year press experiment in which I removed AHM from 50 randomly trees, while leaving 50 as controls. In addition, I marked and followed 30 trees from which AHM were naturally absent. To assess if mesquite quality differed between trees hosting AHM and trees in which AHM were naturally absent, in 2012 I assayed foliar condensed tannin concentrations, a secondary defense compound, and, in 2015, I measured foliar nitrogen, phosphorous, potassium, magnesium, and iron as they are essential for growth and reproduction. I compared the reproductive potential between AHM present and removed trees by counting flowers and fruits across all 4 years of the study. Mesquite that hosted AHM contained significantly less condensed tannins and significantly higher concentrations of N%, Mg, and Fe. Furthermore, over the duration of the study mesquite hosting AHM contained significantly more flowers than those from which AHM were removed or naturally absent. My results indicate that AHM select trees of high quality and their continued presence is associated with high levels of reproductive potential.

Most studies that have evaluated community-level effects of AHM compare total abundance and species richness in communities (or host plants) with and without AHMs. However, both measures are dependent on sampling effort, complicating comparisons across different studies. To examine the effects of AMH on the arthropod community in mesquite, I first compared family richness and alpha diversity using standardized rarefaction and extrapolation curves. I then measured beta diversity and turnover in community composition from one year to the next. The removal of AHM increased invertebrate diversity and significantly altered community composition. Although treatments did not statistically differ in turnover rates, replacements occurred among treatments at the family level which may be biologically meaningful. Furthermore, herbivore and predator populations increased, and pollinator populations decreased following the removal of AHM. These results suggest that the presence of AHM can alter the composition of arthropod communities and food-web dynamics. However, these effects were significant in some years and not others, suggesting the importance of temporal variation in drivers of communities. Overall, my work demonstrates that AHM can be drivers of community composition and illustrate the importance of examining their effects across multiple seasons.
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CHAPTER 1: THE STRUCTURING OF COMMUNITIES THROUGH DIRECT AND INDIRECT INTERACTIONS DERIVED FROM ANT PROTECTION MUTUALISMS: A REVIEW AND PROSPECTUS

1.1. Abstract
Ant-protection mutualisms (APM) are ubiquitous across most natural systems, many agricultural systems, and their efficacy as biotic defenders has been extensively studied. APM act as biotic defenders for a variety of plants (myrmecophytes) and arthropods (myrmecophiles), primarily hemipterans and several species of lepidopteran larvae. The presence of myrmecophytes and myrmecophiles tended by ants are found to reduce the populations of specific herbivores, predators, parasitoids and pollinators. In addition, plants hosting APM also benefit from the presence of tending ants, thus leading to reduced herbivory and increased reproductive potential. Despite the preponderance of pairwise studies testing the efficacy of APM as biotic defenders, the long-term and community level effects of APM has received very little attention. The aim of this paper is to summarize the current understanding of the role APM play as biotic defenders, identify pressing questions that have remained largely unexplored regarding APM, and discuss the importance of addressing these issues to further our understanding of the role APM have on communities. Overall, as protection mutualisms are found in virtually every ecosystem and species interactions do not occur in a vacuum, furthering the current understanding of APM will provide critical insight into how communities are structured and potentially how communities will be affected should APM be lost or introduced.
1.2. Introduction

Many organisms engage in mutualisms in which interacting species exchange services that result in a net benefit for both partners. Benefits may be exchanged directly between partner species in pairwise interactions with no intermediary, i.e. plant-pollinator interactions (Bronstein 1994), or the benefits can be derived indirectly via interactions with a third species (Wootton 1994). For example, the benefits derived from mutualisms involving ants often come from indirect interactions in which ants provide a benefit to either a plant through the removal of herbivores (Styrsky and Eubanks 2010, Stanton and Palmer 2011), or to another arthropod by removing or deterring predators, e.g. protection mutualism (Queiroz and Oliveira 2001, Perfecto and Vandermeer 2006). Protection mutualisms have the potential to have community-wide impacts as they directly and indirectly alter the behavior, foraging ability, and movement of other species in the community. Mutualisms involving ants and herbivores and the host plants on which they occur are some of the most commonly studied terrestrial protection mutualisms. Almost every ant species opportunistically engages in protection mutualisms (Carroll and Janzen 1973) with various species of plants with specialized structures that benefit ant partners (e.g. myrmecophytes; Gaume and McKey 1999, Trager and Bruna 2006) and insects that produce or excrete carbohydrate resources harvested by ants (e.g. myrmecophytes; Kronauer and Pierce 2011, Robinson and Robinson 2013).

Myrmecophytes maintain mutualisms with ants in a variety of ways. Some plants (ca. 681 species) produce modified thorns termed, domatia, to house ants (Chomicki and Renner 2015), and may also feed their ant partners with food bodies rich in lipids (O’Dowd 1982). Over 3,600 species provide carbohydrate rewards through extra floral nectaries (EFNs)-- bowl shaped glands outside the flower that produce nectar for the sole
purpose of attracting ants. The ‘bowls’ are continuously refilled, providing a reliable and important carbohydrate resource for ants (Holway et al. 2002, Lach et al. 2010). In return, ants defend EFNs and their host plants from arthropod and mammalian herbivores (Young and Okello 1998, Goheen et al. 2013). Like extra-floral nectar, honeydew is a renewable and reliable carbohydrate resource harvested by ants in exchange for protection. However, honeydew is excreted by various species of hemipterans and some species of lepidopteran caterpillars (Robinson and Robinson 2013). The availability of honeydew provides ants with an energy source that allows for increased foraging and activity that ultimately, enhances overall colony success (Helms and Vinson 2008). In turn, tending ants defend their honeydew producing partners from predators, parasitoids, and competing herbivores (Del-Claro and Oliveira 1993, Gove and Rico-Gray 2006).

Myrmecophylic and myrmecophytic mutualisms, collectively called “ant-protection mutualisms” (APM), provide ideal systems to investigate the potential for mutualisms drive community composition as ants play important roles as predators, scavengers, and seed dispersers (Carroll and Janzen 1973, Davidson 1997, Helms and Vinson 2002, Rowles and O’Dowd 2009) and engaging in mutualisms can potentially amplify these effects through increased activity and aggressiveness (Davidson 1997, Helms and Vinson 2008). Consequently, the increased activity and aggressiveness in tending ants can then negatively affect the broader community of herbivores (Wimp and Whitham 2001), predators (Kaplan and Eubanks 2005), parasitoids (de Freitas and Rossi 2015), and pollinators (Assunção et al. 2014) that utilize their host plant. The reduction of the invertebrate community may then feedback and indirectly affect their host plant’s reproductive potential and quality (Fischer et al. 2005, Eubanks and Styrsky 2006).

Therefore, we present four pressing questions that have remained largely unexplored regarding APM and discuss the importance of addressing these issues to further our understanding of the role within their communities. First, how does the presence of APM directly and indirectly affect invertebrate communities and do their effects extend spatially? Second, what are the temporal effects of APM on the invertebrate communities? Third, how does the of APM directly and indirectly affect ecological processes; specifically, pollinator behavior and efficacy? Finally, how does the alteration of the invertebrate community by APM affect their host plant’s reproductive potential?

1.3. Community level effects of ant-protection mutualisms

Based on recent meta-analyses of primarily direct, pairwise species interactions with tending ants, APM clearly have the potential to affect how communities are structured through altering the abundances of certain species of herbivores, predators, and parasitoids (Rosumek et al. 2009, Chamberlain and Holland 2009, Trager et al. 2010,
Zhang et al. 2012). For example, arthropod herbivore abundances increased by 50% and 56.6% in the absence of ant-plant mutualisms and ant-hemipteran mutualisms (Rosumek et al. 2009, Zhang et al. 2012). In addition, they found that arthropod predators increased by more than 100% in the absence of tending ants (Rosumek et al. 2009, Zhang et al. 2012). Since most of the studies reviewed by Rosumek et al. (2009) and Zhang et al. (2012) only measured direct effects of APM on herbivore or predator populations, the full effect of APM is not clear as herbivore and predator populations are confounded with one another. One might speculate, for example, that an increase of predators in the absence of ants could be driven by the loss of direct negative interactions with ants (e.g. the removal of an important spider predator; Morse 1985, Mestre et al. 2014) or an indirect positive result due to the increase of arthropod prey in the absence of ants. Conversely, the increase in herbivore populations after the removal of APM may be much stronger but dampened by the subsequent increase in predator populations.

In addition to potentially altering the invertebrate community composition on their host plant, APM may indirectly influence the composition of the invertebrate communities on surrounding plants not hosting APM. Several studies have attributed the decreased diversity in arthropod populations in the presence of APM to predation and removal of arthropods by tending ants (Fowler and MacGarvin 1985, Sakata 1995, Wimp and Whitham 2001, Kaplan and Eubanks 2005, de Freitas and Rossi 2015). Alternatively, the decrease in arthropod diversity in the presence of APM may result from arthropods actively avoiding plants hosting APM (i.e. bruchid beetles; Pringle 2014) and preferentially selecting APM free plants (Grinath et al. 2012) creating a mosaic of differing communities (Wimp and Whitham 2001, de Freitas and Rossi 2015, Nasseri
Therefore, studies need to expand from direct, pairwise interactions to whole community level effects of APM in order to tease out the full potential of APM in structuring communities (Assunção et al. 2014, Palmer et al. 2015).

1.4. Temporal indirect effects of APM on communities

Most studies focusing on the effect of APM on their communities have only measured their effect over the short-term (single season). Although single season studies provide valuable information on the effects APM have on their communities, short-term studies cannot capture the long-term indirect effects the presence of APM have. Recent studies have argued for the importance of examining the long-term effects of APM on invertebrate communities and their host plants (Bilick and Tonkel 2003, Stadler and Dixon 2005, Grinath et al. 2012, Ando et al. 2017). Specifically, extending the time scale over which APM are studied will allow for greater understanding in how host plants and the surrounding invertebrate communities respond to the natural variation in APM populations over time (Bilick and Tonkel 2003). Furthermore, sampling communities over longer periods can provide insight into the long-term indirect effects of APM on communities, even after APM are no longer present (Ando et al. 2017). Early in the season the presence of ants tending aphids reduced the abundance of two major herbivores of tall goldenrod (*Solidago altissima*) and that aphid herbivory promoted regrowth of goldenrod leaves later in the season. The increased leaf growth and the loss of tending ants later in the season then indirectly benefitted grasshopper population (Ando et al. 2017). Thus, indirect effects may persist across time and illuminates the need to extend the temporal scope of APM studies. Focusing on the more long-term studies will not only be beneficial to filling the gaps on the ecological importance of APM but also would be greatly important for other studies carried out in systems that contain
APM. Thus by not accounting for the presence of APM or the potential of indirect temporal effects could confound those results (Ribas and Schoereder 2002).

**1.4. Effect of APM presence on pollinator behavior and efficacy**

Ants are aggressive and thus the use of EFNs that are often close to flowers, and the tending of hemipterans that feed near floral bodies, one might expect APM would negatively impact visitation by pollinators. Indeed the presence of tending ants can reduce pollinator visitation rates (Ness 2006, Assunção et al. 2014, LeVan et al. 2014). However, the expected concomitant reduction in fruit set has not been demonstrated and, where studied, APM presence has resulted in higher fruit set than where APMs are absent (Rosumek et al. 2009, Chamberlain and Holland 2009, Trager et al. 2010). Increases in fruit production may be driven by higher flower abundances in APM present plants (Messina 1981, Holway et al. 2002, Styrsky and Eubanks 2010) and thus leading to higher number of flowers/plant visited in each foraging bout (Conner and Rush 1996, Greenleaf and Kremen 2006) may facilitate an increase in cross pollination (Altshuler 1999). The enhanced pollination effectiveness and delivery of outcross pollen may lead to increased fruit production, larger fruit size and seed abundance (Sapir et al. 2017).

In addition, although pollinators may incur energetic costs of disrupted foraging bouts by tending ants (Assunção et al. 2014), pollinators may also reap a benefit by foraging in predator reduced zones. For example, crab spiders are specialized hunters that wait in flowers, perfectly camouflaged, to ambush floral visitors and significantly impact pollination (Suttle 2003). However, in the presence of tending ants, spider abundances are significantly reduced (Buffington and Redak 1998, Del-Claro and Oliveira 2000, Nasseri 2018). Furthermore, pollinators may preferentially select plants with ant activity not only for foraging but also as oviposition sites (Mota and Oliveira 2016, Wynhoff and
van Langevelde 2017, Carleial et al. 2018). Thus, the presence of APM has the strong potential to directly and indirectly effect pollinator behavior, efficacy, and reproduction and thus the effects of APM on pollinators should be more fully examined.

1.5. Long-term effect of ant-protection mutualisms on the reproductive potential of host plants

In short-term studies, the presence of APM are generally found to have beneficial effects on their host plant’s reproductive potential, e.g. flower and fruit production (Rosumek et al. 2009, Chamberlain and Holland 2009, Trager et al. 2010, Zhang et al. 2012). The protective services afforded host plants by the presence of APM (Ito and Higashi 1991, Del-Claro and Oliveira 1993, 2000, Gove and Rico-Gray 2006, Styrsky and Eubanks 2010) may also indirectly benefit their reproductive potential through shunting resources towards growth and reproduction and away from defense (Nomura et al. 2000, Eck et al. 2001). The defensive benefits accrued by hosting APM, whether they are derived from direct or indirect interactions, have the potential to have long-term effects on host plant reproduction, however they have rarely been tested (Grinath et al. 2012; Nasseri 2018). For example, *Chrysothamnus viscidilorus* directly benefitted from the presence of the ant *Formica obscuripes* tending *Publilia modesta* through significantly reducing the population of harmful chewing herbivores. However, increased herbivory by protected *P. modesta* negatively affected *C. viscidilorus* growth and seed weight the following season (Grinath et al. 2012). Conversely, the removal of an ant-hemipteran mutualism on honey mesquite resulted in significant decreases in flower quantities and overall plant quality (Nasseri 2018). The long-term reduction of herbivory regardless of the driver can significant effects on the reproductive potential of future generations. For example, defoliation of *Primula veris* during the flowering season
negatively affected seed set within that season and reduced growth the following seasons. 

Whereas, herbivory during fruit development led to a decrease in fertility of reproductive individuals the following season (García and Ehrlén 2002). Likewise, the long-term suppression of herbivory, spanning several generations on common primrose, *Oenothera biennis*, found that the sustained reduction in herbivory allowed for earlier flowering and lower investment in secondary defense compounds granting a competitive advantage against unprotected neighboring plants (Agrawal et al. 2012). These studies suggest that the presence of APM have the potential through the suppression and exclusion of herbivores and seed predators to not only affect the current reproductive success of their host plant but their future reproductive potential as well, particularly in long-lived plants.

**1.6. Conclusion: Importance of Incorporating Indirect Interactions in Ecological Studies**

Moving forward, we argue the need for more studies that simultaneously investigate the long-term direct and indirect effects of APM on the overall arthropod community and the response of their host plant over multiple reproductive seasons. The broadening of spatial and temporal scales and extending from pairwise species comparisons to whole community level sampling when studying APM will expand the current understanding of the overall effect APM have in delineating important ecological and evolutionary processes. Especially in systems that contain strongly interacting species or, in the case of mutualisms, interactions that have the potential to be strong drivers with effects that cascade through the community (Wootton 1994). Understanding indirect interactions can also have significant unexpected consequences. Therefore, by expanding our knowledge on how indirect interactions cascade through communities and
the unexpected consequences they may have, we can better understand the mechanisms for how they drive community composition (Palmer et al. 2015).
1.7. Reference


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CHAPTER 2: AN EFFECTIVE AND EFFICIENT METHODS OF REMOVING AND SAMPLING ANTS OVER LARGE SPATIOTEMPORAL SCALES

2.1. Abstract
Ants are ubiquitous across tremendously diverse habitat types and can be important drivers of the communities and ecosystems in which they are found. Understanding their role is best achieved by removal studies, but finding effective means for removing ants can be difficult. Ant removal studies focusing on arboreal communities are often carried out using sticky barriers that are not logistically efficient for large-scale or long-term studies. In addition, methods for sampling arboreal ants are cumbersome and logistically difficult to implement. Here I describe an efficient and cost-effective method that allows for long-term ant removal and sampling in arboreal communities over large-spatial scales. The method can be utilized in remote areas with easily accessible materials and can be done by a single researcher in the field. The procedure can also be modified to fit any habitat type as well as modified to remove certain species of ants depending on body size. Using this method, I was able maintain a press ant removal experiment on honey mesquite, *Prosopis glandulosa*, over a three-year study.
2.2. Introduction

Ants are ubiquitous across natural, cultivated and urban landscapes. With over 12,000 species worldwide ants have been shown to fill important niches across all trophic level. Ants alter soil chemistry through nest building activities, act as important seed dispersers, and are often key predators capable of altering arthropod communities through direct removal of prey and suppression of other arthropod predators (Cheng et al. 2015; Torra et al. 2016). Furthermore, many ants form mutualistic partnerships with host plants or with other arthropods. These partnerships can alter numerical or behavioral dominance of other species within a system. Given their ubiquity and functional importance, understanding the role of ants in communities has been the focus of much research (Styrsky and Eubanks 2007). To understand the effects of ants on community dynamics, most studies undertake ant removal experiments.

However, maintaining ant removal treatments and sampling of ants, especially arboreal species, is cumbersome and logistically difficult especially for long-term studies. Thus, most removal experiments are short-term, pulse experiments implemented only once (Bender et al. 2016). Short-term, pulse experiments provide useful information in understanding the immediate effects of ants on communities, but only provide a snapshot of the impact ants may be having. Establishing methods that allow for long-term studies in which ants are continuously removed for the duration of the project (e.g. press manipulations) provides a much more detailed understanding of the impact of ants.

Ant removal treatments in arboreal systems have been primarily conducted through the application of canopy fogging with harsh pesticides (Stanton and Palmer 2011; Stork et al. 2014), using smoke to drive out ants (Palmer and Brody 2013) or by hand (Palmer et al. 2002; Stanton et al. 2002). With the exception of hand removals,
these methods are applied broadly thereby exterminating non-target species, are extremely expensive (Häuser and Riede 2015), require specialized equipment (Stork et al. 2014) or are logistically difficult especially in large-scale experiments. Furthermore, after application of these methods, experimenters must employ an exclusion barrier (e.g. tanglefoot), to prevent recolonization (Bartimachi et al. 2015). There are logistical problems with sticky barriers, as they require regular maintenance or reapplication to maintain impermeability and they kill many non-target species including other arthropods and even small reptiles (Sanchez and Bellota 2015). Plastic cylindrical pipes filled with ant attractants (canned tuna fish) mixed with various ant poisons have shown to successfully remove arboreal ants in New Guinea rainforests (Klimes et al. 2011). Using this method, Klimes et al. (2011) removed ca. 82.4% of ants per station in primary forest and 91.2% in secondary forest. However, a separate system needs to then be installed to sample the ant community.

Another difficulty with investigating the ecological impacts of ants is efficiently and systematically sampling ants. Sampling terrestrial ants using pitfall traps buried into the substrate (Schmidt and Solar 2010) is considerably easier than sampling arboreal ants. Some studies have successfully utilized a similar approach for arboreal sampling through attaching vials to branches by sliding them between vines (Samson et al. 1997) or affixing the vials directly to branches or the trunk (Kaspari 2000).

Kaspari (2000) designed an ingenious method of establishing an arboreal pitfall system. He used a modified slingshot equipped with fishing reels, canvas and test tubes (see Kaspari 2000 for full methods). Using a slingshot, a lead weight attached to fishing line was launched over the focal branch. The fishing line was then replaced with nylon
rope attached to canvas material that held a vial acting as a pitfall. The nylon rope, anchored to the ground, could be used to raise and lower the pitfall traps. However, this method would only be feasible on trees with a very open canopy without many low hanging branches, and carries a risk of injury from the lead weight ricocheting out of the slingshot when fired (Kaspari 2000). In addition, it requires a team of people to successfully install baits over an area large enough to provide ecologically relevant results (Klimes et al. 2011).

Here, I describe a low cost, low maintenance system that allows one to efficiently remove arboreal ants while simultaneously sample them through combining and modifying the methods of Klimes et al. (2011) and Kaspari (2000). My modified design utilizes easily obtainable, low cost materials that can be installed in any tree or shrub to continuously remove ants over a long-term while minimizing exposure of poison to non-target species. The bait system I describe can then easily be converted to sample ants in one simple step. I successfully utilized this system to continually remove and sample ants in a three-year press experiment investigating the ecological effects of ants on honey mesquite (*Prosopis glandulosa*) in south Texas.

2.3. Materials and Methods

2.3.1. Ant bait stations

Ant bait stations were constructed out of PVC pipes (6.35 mm diameter). The PVC was cut to 153 mm long segments using a hand-held hacksaw. A power drill with 2mm drill bit was used to drill entrance holes down the length of PVC and into the opposing wall. These holes allow ants to freely enter and leave while minimizing exposure of the poison to non-target species and protecting the baits from rain and wind that can wash away, leach or spread the poison into the surrounding matrix (Fig. 2.1).
The ends of the PVC pipe were capped with Great Stuff™, a foam sealant. After hardening, the foam can be removed like a cork when bait stations need to be refilled. Bait stations were filled with poison granules comprised of 0.88% hydramethylnon. Hydramethylnon has a delayed toxicity which allows worker ants to ingest the poisoned granules, return to the colony and disperse the poison baits to nest mates through trophollaxis. The baits are long-lasting, and in my study, bait stations were refilled every four to five months.

2.3.2. Arboreal Pitfall Traps
Arboreal pitfall traps were constructed from 50.0 mL centrifuge tubes. The interior walls of the tubes were coated with a silicon lubricant to prevent ants from climbing out once inside the tube. Entrance holes were drilled into the center of the centrifuge tubes’ cap using a 2.0mm drill bit. Centrifuge tubes were filled with 25mL of a 25% sugar solution. A tube was placed in each opening cut into the canvas bridge and were checked every 48 hr (Fig. 2.2).

2.3.3. Bridge construction
Plastic zip ties connected heavy duty canvas drop cloth to opposite branches forming a ‘bridge’. The canvas material was cut into ca. 115 mm strips with a small incision made down the middle of each end. The incision allowed the canvas to naturally wrap around the branch forming a natural connection between the canvas and the branch (Fig. 2.1). Slits should be cut near the ends of the now affixed canvas, at a minimum of 65 mm apart and just long enough to slide the 50.0 mL centrifuge tube through (ca. 25 mm in length). The slits serve a dual purpose as 50.0 mL centrifuge tubes can be inserted into each slit to act as arboreal pitfall traps to sample ants as well as a means of securely attaching the PVC bait traps. Centrifuge tubes placed in the openings need to be flush
with the canvas and the ends of the PVC can slide in and be held in place on the now constructed bridge (Fig. 2.1).

2.3.4. Experimental design

The study was conducted at the Rob and Bessie Welder Wildlife Refuge Foundation (WWRF) in south Texas (28°06’31.78”N, 97°23’56.15”W). WWRF is ca. 7,800 acres and managed for wildlife and rangeland experiments. Located in the Gulf Coastal Plain of south Texas, WWRF consists of xeric, grassland-scrub savannas dominated by mesquite. At WWRF, the four most common genera of ants are *Camponotus* sp., *Crematogaster* sp., *Dorymyrmex* sp., and *Solenopsis* sp. At the landscape level, more than 85% of mesquite hosted *Camponotus sayi*. *Camponotus sayi* nests in fissures in the trunk at the base of mesquite and move onto the branches to forage and harvest honeydew produced by treehoppers.

To determine the efficacy of the removals, and the subsequent effect of ants on the mesquite arthropod community, I employed the above-described baits starting in May 2013. I haphazardly selected 100 focal trees >2.5 m in height (4.13 ± 0.11 m) containing ants. Trees were determined to host ants if I observed two independent aggregations of ants foraging in the canopy within a five-minute scan. I permanently marked all trees with numbered aluminum tags and recorded their GPS location. From the 100 selected trees, I randomly selected 50 trees from which to remove ants and 50 to act as control trees. Ants were sampled using pitfall traps and vacuum sampling.

The efficacy of the ant baits in removing and preventing recolonization was determined by comparing ant abundances using both sampling methods prior to and six weeks after application of ant baits (June and August 2013, respectively). Pitfall traps
were only installed in trees assigned to the ant removal treatments. Therefore, to confirm that changes in ant abundance were an outcome of establishing the bait stations and not due to disturbance by traps or natural changes in colony size across the 6-week interval, I vacuum sampled both ant-removal and control trees and compared ant abundance in June 2013 versus August 2013.

2.3.5. Pitfall traps
Pitfall traps were installed in the 50 ant removal treatment trees and baiting began in June 2013. Each bridge contained two pitfall traps with three bridges installed per tree (Fig. 2.2). Pitfalls were left open for 48h. After 48h, the pitfall traps containing ants were collected and transferred to vials for quantification. Pitfall traps were removed after 48h and replaced with a bait station containing Hydramethylnon. Ant bait stations remained in the trees and were refilled in late August and December of 2013, 2014, and 2015. Installation of bridges took approximately one hour for a single researcher to install per tree. Collection and transfer of pitfall samples (six per tree) to vials and replacing with filled bait stations (three per tree) took approximately 10 minutes for a single researcher.

2.3.6. Vacuum sampling
Vacuum sampling provided a pre-treatment baseline for both the number of ants in trees as well as the number, diversity and identity of the entire arboreal arthropod community. All trees were vacuumed sampled in June from 2013 – 2015. Trees were vacuumed continuously for six minutes between 800 h and 1200 h in 2013 and 2014. In 2015, due to time constraints, sampling was reduced to three minutes.

2.3.7. Data analysis
To assess the efficacy of ant baits in removing ants, I used a matched pairs analysis with a Wilcoxon-Signed Ranks Test to test ant abundances prior (June 2013) and
post (August 2013) establishment of the bait stations (JMP Pro 12.1.0). As the data did not meet the assumptions of normality, Wilcoxon-Signed Ranks Tests were performed to determine if there were any significant difference in ant abundances between ant removal treatments and control treatments prior and post application of bait stations. All results are presented as mean ± SD. Since, pitfalls were only established in ant removal treatment trees, pitfall abundances were only available to those trees. However, vacuum sampling was carried out on all trees over the same sampling period, so abundances were also compared between trees prior to ant removal and after ant removal. To ascertain if there were differences between the two sampling techniques, I compared the percentage change in mean ant abundance after the introduction of the bait stations.

2.4. Results

2.4.1. Pitfall traps
The administration of the poison bait stations resulted in an 87.4% reduction in mean ant abundance in ant removed treatments. In June 2013 there was an average of 130.9 ± 98.0 ants/tree prior to treatment application. Six-weeks after the treatment application, August 2013, mean total ant abundance significantly dropped to 16.5 ± 35.1 ants/tree (Z = 7.46, p = <0.0001).

2.4.2. Vacuum sampling
There was no significant difference in ant abundance between trees assigned to ant removal and control trees prior to the ant removal application (mean ants/tree: 17.1 ± 14.5, 18.3± 16.6, respectively; Z = 0.4728, p = 0.6363; Fig. 2.3, Table 2.1). Similar to pitfall trapping, there was an 85.3% decrease in mean ant abundances in ant-removal trees when vacuum sampled six-weeks later. Mean ant abundance significantly dropped from 17.1 ± 14.5 ants/tree in June 2013 to 2.6 ± 3.4 ants/tree in August 2013 (Z = 6.929,
p = <0.0001; Fig. 2.3), whereas control trees harbored 18.3 ± 16.6 ants/tree in June 2013 and 14.6 ± 9.8 ants/tree in August 2013 (Z = 0.7753, p = 0.4382). In August 2013, there were significantly fewer ants in the ant removal trees than in the control trees (Z = 7.1904, p = <0.0001, Fig. 2.3). Trees were vacuumed sampled again in June 2014 and June 2015. Ant abundances were significantly higher in control trees than in ant removal trees in 2014 and 2015 (Table 2.1).

2.5. Discussion

The ability to effectively and efficiently remove ants is critical for experimentally determining their role in communities and ecosystems. My results demonstrate that the ant baits, modified from those designed by Kaspari (2000), can be effectively, efficiently and inexpensively used to remove and sample arboreal ants. The bait stations were not only effective in removing ants, they were efficient to install and collect as pitfall samples. Similar methods require significantly more person-hours and greater expense (Kaspari 2000; Klimes et al. 2011).

Although I only used the pitfall traps at the start of the study to determine the efficacy of the bait stations, vacuum sampling provided a sufficient subsampling of the ant community. If my objective had been to simply determine the abundance of ants in mesquite trees pitfall traps would have been the most efficient means of sampling. However, pitfall trapping may not capture the diversity of ants inhabiting a tree as the foraging behavior (and thus probability of landing in a pitfall trap) will differ among species. Vacuum sampling thus provides a way to sample a broader representation of ant taxa on mesquite.

Understanding the role ants play in driving communities and ecosystems is especially important with the increasing occurrence of invasive ants in many systems
(Holway et al. 2002; Lach 2003; Ness and Bronstein 2004). The methodologies described here and implemented in the field provide a novel, inexpensive and logistically efficient way to establish long-term ant removal treatments as well as sampling ant abundance and diversity in arboreal communities.
2.6. References
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Stanton, M.L., Palmer, T.M. and Young, T.P. (2002). Competition-colonization trade-


2.7. Table Legend
Table 2.1: Mean ± standard deviation (SD) ant abundance through vacuum sampling between treatment trees from June 2013-2015. Comparisons were made of ant abundances within year not between years.
Table 2.1. Mean ± standard deviation (SD) ant abundance through vacuum sampling between treatment trees from June 2013-2015. Comparisons were made within year between treatments not between years.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>Mean ± SD</th>
<th>Z score</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>AHM Present</td>
<td>18.3 ± 16.6</td>
<td>Z = 0.4728</td>
<td>0.6363</td>
</tr>
<tr>
<td></td>
<td>AHM Removal</td>
<td>17.1 ± 14.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>AHM Present</td>
<td>19.1 ± 16.6</td>
<td>Z = 6.3811</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>AHM Removal</td>
<td>3.1 ± 6.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>AHM Present</td>
<td>8.9 ± 14.5</td>
<td>Z = 5.3611</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>AHM Removal</td>
<td>2.1 ± 4.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In 2015, trees were sampled for three minutes instead of six minutes as in 2013 and 2014.
**2.8. Figure Legend**

Figure 2.1: Bridges constructed from heavy duty paint canvas drop cloth containing an arboreal ant bait station affixed to branches on honey mesquite (*Prosopis glandulosa*).

Figure 2.2: Pitfall trap with sugar solution placed within the bridges constructed from heavy duty paint canvas drop cloth affixed between branches in honey mesquite (*Prosopis glandulosa*).

Figure 2.3: Ant abundances sampled through (A) Pitfall trapping; Pre = prior to the application of ant poison bait stations, Post = six-weeks post application of ant poison bait stations. (B) Ant abundances sampled via vacuum sampling over three years (2013-2015); 2013 ant abundances were conducted prior to the application ant poison bait stations.
Figure 2.1. Bridges constructed from heavy duty paint canvas drop cloth containing an arboreal ant bait station affixed to branches on honey mesquite (Prosopis glandulosa).
Figure 2.2. Pitfall trap with sugar solution placed within the bridges constructed from heavy duty paint canvas drop cloth affixed between branches in honey mesquite (Prosopis glandulosa).
Figure 2.3. Ant abundances sampled through (A) Pitfall trapping; Pre = prior to the application of ant poison bait stations, Post = six-weeks post application of ant poison bait stations. (B) Ant abundances sampled via vacuum sampling over three years (2013-2015); 2013 ant abundances were conducted prior to the application ant poison bait stations.
CHAPTER 3: LONG-TERM EFFECTS OF ANT-HEMIPTERAN MUTUALISM ON THE REPRODUCTIVE POTENTIAL OF HONEY MESQUITE (PROSOPIS GLANDULOSA)

3.1. Abstract
Mutualisms involving ants and hemipterans have recently been identified as keystone interactions due to their ability to structure and alter local communities. Ants tending hemipterans may actively defend against all intruders and thus negatively affect the arthropod community co-inhabiting their host plants. These interactions may indirectly benefit the host by reducing herbivory, and creating a potential feedback loop. However, the long-term effects of ant-hemipteran mutualisms (AHM) on their host plants are poorly understood. Here we investigated the long-term effects of AHM on the quality and reproductive effort of honey mesquite, Prosopis glandulosa Torr., at Welder Wildlife Refuge in south Texas. We established and maintained an AHM removal experiment from 2013-2016 on 130 mesquite trees (N_{AHM present} = 50, N_{AHM removed} = 50, N_{AHM absent} = 30). To determine the relationship between AHM and mesquite quality, we assayed condensed tannin concentrations in 2012, then in 2015 we measured nitrogen (%N), phosphorous (P), potassium (K), magnesium (Mg), and iron (Fe) in mesquite leaves and reproductive effort was quantified by counting flowers and fruits. AHM populations were significantly associated with higher quality mesquite and the long-term presence of AHM did not negatively affect mesquite quality (MANOVA: F_{2,104} = 2.95, P > 0.01). The presence of AHM were also found to have positive long-term effects on mesquite reproductive effort, specifically flowers (repeated measures GLMM: Wald x^2 = 9.85, P < 0.01). Our results indicate that mesquite quality drives the establishment and maintenance of AHM presence, however, mesquite are then dependent on the presence of AHM to maintain high levels of reproductive effort and quality. Altogether, these findings provide evidence for a positive feedback loop between AHM and their host plant.
3.2. Introduction

Ants play important roles in structuring communities (Styrsky and Eubanks 2007, Grinath et al. 2012, Ando et al. 2017). Their effects as mutualists, acting as body guards and aggressively defending their myrmecophilous partners, have been well studied (Styrsky and Eubanks 2007, Rosumek et al. 2009, Chamberlain and Holland 2009, Trager et al. 2010, Zhang et al. 2012). Less well known are their indirect effects on host plants mediated through their tending of herbivores.

Approximately half the ant genera in the world (41%) form a classic food-for-protection mutualism with hemipterans (Oliver et al. 2008). Hemipterans produce honeydew, a reliable and renewable source of carbohydrates and amino acids, as a food reward for ants. In exchange, ants aggressively defending hemipterans from predators and parasitoids. Ants tending hemipterans may also target and remove non-honeydew producing herbivores from their host plants (Del-Claro and Oliveira 1993, Gove and Rico-Gray 2006). Thus, AHM may positively affect their host plants. Ants tending hemipterans may thwart other herbivores and, as a result, reduce herbivory. Over the long term, the presence of AHM may allow plants to reduce investment in defense (Nomura et al. 2000, Eck et al. 2001) and allocate more resources into and growth and reproduction (Rosumek et al. 2009, Chamberlain and Holland 2009, Trager et al. 2010). The presence of AHM may also protect a host plant’s reproductive effort by reducing florivory and seed predation (Ito and Higashi 1991, Del-Claro and Oliveira 2000, Styrsky and Eubanks 2010). Conversely, any defensive benefits accrued by hosting AHM would be negated if herbivory by tended hemipteran populations reduces their host plant reproductive effort (Denno et al. 1995, Stadler et al. 2002, Styrsky and Eubanks 2007). In addition, the
presence of tending ants can deter pollinators and subsequently reduce pollination efficiency and fruit set (Lach 2007).

While providing insight into the possible effects of AHM on their hosts, these studies have most often encompassed a single reproductive season (but see Mooney 2007, Grinath et al. 2012). Because the presence and abundance of AHM vary temporally (Bilick and Tonkel 2003) and so do the effects they have on their host plant (Grinath et al. 2012, Ando et al. 2017), longer term studies are needed. For example, early in the season the presence of ants tending aphids reduced the abundance of two major herbivores of tall goldenrod (*Solidago altissima*). However, regrowth of goldenrod leaves later in the season due to earlier herbivory by tended aphids resulted in higher abundances of grasshoppers when AHM were no longer present (Ando et al. 2017). Furthermore, the presence of AHM can impact future reproductive seasons either by reduced levels of herbivory due to herbivore suppression or increased herbivory from protected hemipteran populations. For example, although the presence of the ant *Formica obscuripes* tending *Publilia modesta* on a host plant, *Chrysothamnus viscidilorus*, resulted in significant reductions of harmful chewing herbivores, but increased abundances of *P. modesta* resulted in lower host plant growth and seed weight the following season (Grinath et al. 2012). Therefore, understanding the long-term effect the presence of AHM have on their host plant requires multi-season empirical studies (Grinath et al. 2012; Ando et al. 2017).

Here, we investigated the long-term effects of AHM on the quality and reproductive effort of honey mesquite, *Prosopis glandulosa* Torr., a long-lived dominant hardwood, in south Texas. To evaluate the long-term effect of AHM on their host plant,
we measured mesquite quality and reproductive effort using a combination of natural surveys (2012) and long-term experimental manipulations (2013-2016) to address three major questions: (1) Does host plant quality acts as a driver for establishing and maintaining strong AHM? (2) Does the presence of AHM negatively affect host plant quality? (3) How does the long-term removal of AHM affect their host plants’ reproductive effort? We hypothesized that hemipterans are choosy about where they reside, and thus we predicted that mesquite hosting AHM populations would be of higher quality than those not hosting AHM. We also hypothesized that the presence of AHM are beneficial to mesquite quality and reproduction. Therefore, we predicted that the long-term removal of AHM from mesquite would result in reduced quality, and lower quantities of flowers and fruits. To address the first question, we surveyed mesquite in 2012 to first determine the prevalence of mesquite hosting AHM and if mesquite hosting AHM differed in reproductive effort and quality from mesquite that naturally did not host AHM. To address question two, we established long-term AHM removal treatments in 2013 and maintained those treatments until 2016. In addition, we included mesquite naturally lacking AHM in 2014 to examine the difference in reproductive effort and quality in naturally AHM absent mesquite to experimentally removed AHM mesquite.

3.3. Methods
3.3.1. Study System
We conducted our study at the Rob and Bessie Welder Wildlife Refuge Foundation (WWR) in south Texas (28°06‘31.78”N, 97°23‘56.15’’W). WWR is ca. 3,160ha and managed for wildlife and rangeland experiments. Located in the Gulf Coastal Plain of south Texas, WWR consists of xeric, grassland-scrub savannas dominated by honey mesquite. Victorian clay, intermixed with sandy loam, comprises the
predominant soil type of WWR. The area experiences a bimodal peak in rainfall with most rainfall occurring in September and May-June (Glasscock et al. 2005).

Mesquite is a nitrogen-fixing legume native to the Americas (Laing et al. 2000). Mesquite is invasive in the Panhandle of Texas and in Mexico and Arizona where it has replaced native live oaks as the dominant hard wood. It is a significant problem as it forms dense, impenetrable thickets resulting in the degradation of pasture and loss of native grasslands (Fulbright et al. 1990, Griffith et al. 2004). In areas where water is scarce, mesquite grows with a shrub-like, highly branched architecture. Mesquite reproduces bi-annually, with a short bloom in March-April followed by an extended reproductive season from May – September; peak flowering occurs in June and July (McGrath et al. 2009). Its flowers are perfect and are produced on yellowish racemes that produce large amounts of pollen. Fruits (beans) mature from July through September and are an important food source for a variety of mammals, birds, and insects (Vines 1960).

At WWR, mesquite hosts *Vanduzea segmentata*, a non-native treehopper which is tended by a native carpenter ant, *Camponotus sayi*. Records indicate that *Vanduzea segmentata* is native to Central America, however little is known of their origin and life history. At WWR, *V. segmentata* aggregates at the base of flowering racemes or seed pods of mesquite and their emergence appears to be strongly tied to the onset of mesquite flowering. The adults are sexually dimorphic; females are larger and greener in color than the smaller, more brown males, and larvae go through five instars before reaching adulthood. The tending ant, *Camponotus sayi*, nests at the base of mesquite trunks in fissures beneath the bark or in dead branches. *Camponotus sayi* workers forage for invertebrate prey and harvest honeydew produced from *V. segmentata* along the
periphery of mesquite branches. Although usually monopolized by *C. sayi*, *V. segmentata* is sometimes tended by three other genera of ants (*Crematogaster* sp., *Dorymyrmex* sp., and *Solenopsis* spp.; personal observation).

### 3.3.2. Natural Surveys, 2012

#### 3.3.2.1. Utilization of mesquite by cattle

WWR was established for rangeland experiments and this hosts small herds of cattle. Cattle periodically browse on mesquite leaves and fruits, but they predominantly use mesquite as resting spots during the hottest part of the day, 12:00h – 16:00h (personal observation). During these periods, the cattle urinate and defecate, providing a concentrated source of raw manure at the base of mesquite trees that may be absorbed by them. Other studies have found that the addition of fertilizer treatments are beneficial to the plants but also to AHM (Strauss 1987). Therefore, to examine if the use of these trees by cattle, and the input of nutrients from dung deposition, altered the reproductive efforts and patterns of use by AHM, we counted cattle dung piles from the base of the trunk to one meter away from the canopy.

#### 3.3.2.2. Prevalence of mesquite hosting AHM

In the summer of 2012, we haphazardly sampled 120 mesquite trees >2.5m in height (4.58 ± 0.86m) to determine the prevalence of AHM present on mesquite. A single observer (N. Nasseri) performed visual scans of the apical stems and flower buds of focal trees for five minutes and scored each tree as: “Present” if at least two separate aggregations of ants tending *V. segmentata*; “Ant only” ants but no aggregations of *V. segmentata*; “Absent” if the mesquite lacked both ants and *V. segmentata*. We never observed untended aggregations of *V. segmentata* in mesquite. We calculated prevalence as a percentage of the number of mesquite in each category over the total number of mesquite surveyed.
3.3.2.3. Quality of mesquite hosting AHM

As a proxy for host quality, we measured condensed tannin (CT) levels. Plants produce CT as a chemical defense which may negatively affect treehopper reproduction and honeydew production (Grayer et al. 1992, Ossipov et al. 1998, Goverde et al. 1999, Awmack and Leather 2002, Stam et al. 2014). Therefore, we used CT as an indicator of mesquite quality. To measure CT, we randomly collected ca. 200g of leaflets 1.5 m above the ground from each tree (N = 120) and dried the leaflets at 50°C for 48h in a drying oven. We extracted condensed tannins using a butanol-HCL-ferric reagent and report condensed tannins levels as leucocyanidin equivalents (%LE) (Porter et al. 1986).

3.3.2.4. Reproductive effort of mesquite hosting AHM

During the peak reproductive period in June (McGrath et al. 2009), we quantified flower and fruit production as a measure of reproductive effort. We divided the canopy into four quadrants based on the cardinal directions and randomly selected the north-west (N-W) quadrant from which to count all flowers and fruits. We then multiplied the counts by four to estimate total flower and fruit abundance.

3.3.3. Experimental manipulation of AHM presence, 2013-2016:

3.3.3.1. AHM removal

To examine the long-term effect the presence of AHM had on their host plant, we established and maintained a fully factorial AHM removal and cattle exclusion experiment from May 2013 through June 2016. In May 2013, we selected 100 trees >2.5m in height (4.13 ± 0.11m) with non-overlapping canopies hosting AHM. Trees were then randomly assigned to an AHM removal treatment (“Present”/“Removed”) and Cattle exclusion treatment (“Unfenced”/“Fenced”; N = 25 trees per treatment combination). To exclude cattle, we erected wire fencing around individual mesquite trees assigned to cattle exclusion treatments, providing ca. 2.5 m buffer. In 2014, we incorporated an
additional 30 mesquite trees that naturally did not host AHM ("Absent") to determine if mesquite that naturally did not have AHM differ from mesquite hosting AHM ("Present") and mesquite with AHM experimentally removed in flower and fruit production and quality over multiple years.

We removed ants from trees assigned to “Removed” as a press experiment over 4 years (Yodzis 1988). Ants were removed with commercially available granular ant poison baits. We installed three bait stations per tree. We constructed bait stations from 15 cm long, 6.35 mm wide polyvinyl chloride (PVC) pipe with removable caps at each end and filled with commercially available ant poison-baits. Along the length of the PVC, 2 mm holes were drilled to allow access by ants and reduce contact by non-target species (Klimes et al. 2011; Nasseri 2018). We maintained the treatments by recharging stations with fresh bait every six months to prevent ants from recolonizing AHM removed trees. In each year, we monitored all trees for AHM presence and absence monthly during the reproductive season to ensure treatment effectiveness (Nasseri 2018).

3.3.3.2. Long-term effect of AHM removal on mesquite quality
To assess the relationship between the presence of AHM and mesquite quality, in 2015 we assayed nitrogen (%N), phosphorous (P), potassium (K), magnesium (Mg), and iron (Fe) in mesquite leaves. These elemental nutrients are required for plant growth, photosynthesis, and reproductive success (Leghari et al. 2016, Razaq et al. 2017). In addition to being critical plant nutrients, they are crucial for enzymatic activity, larval development, reproduction, and honeydew quality in hemipterans (Strauss 1987, Silva and Uchida 2000, Cisneros and Godfrey 2001, Stadler et al. 2002, Gove and Rico-Gray 2006). We conducted our assays on young leaves from 113 mesquites trees (AHM Present = 48; AHM Removed = 50; AHM Absent = 15). We randomly collected ca. 200
g of leaflets 1.5 m above the ground from each tree. Elemental nutrient analyses were conducted by the Texas AandM University AgriLife Soil, Water and Forage Testing Laboratory in College Station, TX. Nitrogen concentration was measured as percent N (%N) and N, P, K, Mg, and Fe concentrations were measured as parts per million (ppm).

3.3.3.3. Long-term effect of AHM removal on mesquite reproductive effort
In 2013, prior to establishing either AHM removal and cattle exclusion treatments, we counted flower and fruit production to confirm that treatments did not differ at the onset of the study and to establish a pre-treatment baseline. Flower and fruit production was subsequently quantified each summer from 2013-2016 in June following the same counting procedure as in 2012.

3.3.4. Statistical Approach
3.3.4.1. Effect of AHM on mesquite quality
To test the effects of AHM removal, fencing and the interaction of both treatments on all the macronutrients, we conducted a two-way multivariate analysis of variance (MANOVA). Macronutrients concentrations were first log transformed to satisfy parametric assumptions of normality and heterogeneity. A significant MANOVA effect resulted in separate univariate analyses of variance (ANOVA) test for each response variable to test for AHM, fence, and AHM by fence interaction. A Tukey Kramer multiple comparison post hoc test was administered to any significant univariate result to determine treatment level effects. The MANOVA was conducted in R using the function “manova” and to test the significance of the MANOVA model and to conduct the univariate ANOVA, we used the function “summary.aov” in base R (R Core Team 2017). We used the procedure describe previously to conduct the Tukey Kramer post hoc test.
3.3.4.2. Effect of AHM on mesquite reproductive effort

To determine the effect of AHM on mesquite reproductive effort we performed a series of generalized linear mixed effect models (GLMMs) using the statistical software R v3.4.0 (R Core Team 2017). GLMMs allow for the use of random effects through linear mixed models combined with the power of generalized linear models that allow customizing family distribution types (i.e. negative binomial, Poisson, etc.) (Bolker et al. 2009). We first fit our GLMMs to a Poisson distribution with a log link using the function glmer in the R package “lme4” (Bates et al. 2015) and tested for overdispersion (>1.4) using the function dispersion_glmer in the R package “blmeco” (Korner-Nievergelt et al. 2015). To correct for overdispersion, we refit the models to a negative binomial distribution (Zuur et al. 2009) using the glmer.nb function in lme4 (Bates et al. 2015). GLMMs fitted to a negative binomial were not overdispersed (<1.4), and we used the function Anova in the “car” R package (Fox and Weisberg 2011) to calculate the Wald chi-square (Wald $\chi^2$) value to test for significance of the fixed effects (Bolker et al. 2009). Tukey Kramer post hoc comparisons were carried out using the function emmeans (R package “emmeans”; Lenth 2018) and cld (R package “multcompView”; Graves et al. 2015) that back-transformed intervals from the log scale and calculated adjusted P values ($\alpha = 0.05$) (Mangiafico 2018). Unless specified, for all GLMMs: AHM treatments (“Present”/“Removed”/“Absent”) and Cattle treatments (“Unfenced”/“Fenced”) were identified as fixed effects, trees were treated as subjects (replicates) within treatment within years as random effects, and years as the repeated factor.

To determine how AHM present mesquite differed from naturally AHM absent mesquite in flower and fruit quantities, we performed GLMMs with natural treatments (“AHM present”, “only ants”, and “absent”) as a fixed effect, tree nested within natural
treatments as a random effect, and abundance of cattle dung as a covariate. To quantify if plants that hosted AHM differed in condensed tannins, we used a linear model (function `lm`, R package: “MASS”; (Venables and Ripley 2002)) with condensed tannins levels expressed as leucocyanidin equivalents (%LE) as the response variable, natural AHM occurrences (“AHM Present”/“AHM Absent”/“Ant Only”) as a fixed effect, and cattle dung abundance as a covariate. Prior to fitting the model condensed tannin measurements had to be log transformed to meet parametric assumptions of normality and homogeneity.

To determine the long-term effect of AHM presence on mesquite reproductive effort we used a repeated measures GLMM on only AHM treatments (“Present”/“Removed”) and Cattle treatments (“Unfenced”/“Fenced”) as fixed effects, trees were treated as subjects (replicates) within treatment within years as random effects, and years as the repeated factor. To examine if trees that naturally hosted AHM differed from those that did not, and to further explore the effects of AHM removal, we compared flower and fruit quantities between AHM treatments (“Present”/“Removed”/“Absent”) from 2014 – 2016. We excluded trees that were fenced in our analysis as we did not fence any of the AHM absent trees. To measure the difference in reproductive effort we fit the same repeated measures GLMM used previously.

All figures were created using the package “ggplot2” (Wickham 2009) and all descriptive statistics are presented as mean ± standard error (SE).

3.4. Results

3.4.1. Natural surveys, 2012

3.4.1.1. Quality of mesquite hosting AHM

Mesquite hosting AHM differed significantly in quality from mesquite naturally lacking AHM, but not mesquite containing only ants ($F_{2,113} = 3.2$, $P < 0.05$, Tukey-Kramer post hoc; Fig. 3.1). Specifically, CT concentrations were significantly lower in
AHM present mesquite (12.8 ± 0.8 %LE) than AHM absent mesquite (20.7 ± 2.4 %LE; Fig. 3.1). Likewise, CT concentrations significantly decreased as dung density increased (F1,113 = 12.2, P < 0.001). However, there was a significant interaction between AHM presence and cattle dung density with CT concentrations only negatively correlating with dung densities on mesquite either not hosting AHM or only having ants (F2,113 = 6.6, P < 0.01; Fig. 3.2).

3.4.1.2. Reproductive effort of mesquite hosting AHM

Of the 119 mesquite trees sampled in 2012, 60.5% hosted AHM, 16.9% hosted only ants and 22.6% had no ants. None of the trees sampled contained only V. segmentata. Flower quantities on mesquite hosting AHM (149.4 ± 14.1) were significantly higher than mesquite naturally absent of AHM (72.8 ± 10.4) or only having ants (89.8 ± 22.5; Wald x² = 10.3, P < 0.01, Tukey-Kramer post hoc; Fig. 3.3). Mesquite flower quantities were also significantly positively correlated to dung densities (R² = 0.16, Wald x² = 11.7, P < 0.001; Fig. 3.5a), and there was no significant interaction with AHM presence and dung densities regarding flower quantities (Wald x² = 2.9, P < 0.05). Mesquite fruits were also significantly more abundant in the presence of AHM then when AHM were either absent or only ants were present (Wald x² = 40.1, P < 0.0001, Tukey-Kramer post hoc; Fig. 3.4), but there was no relationship in fruit abundance and dung densities (R² = 0.01, Wald x² = 1.21, P > 0.05; Fig. 3.5b) nor was there a significant interaction between AHM presence and dung densities (Wald x² = 2.1, P < 0.05).

3.4.2. Experimental removal of AHM, 2013-2016

3.4.2.1. Long-term effect of AHM removal on mesquite quality

The 2-way MANOVA indicated that macronutrients concentrations were significantly affected by AHM treatments (F2,104 = 2.95, P > 0.01), but not fencing (F1,104 = 0.812, P < 0.05), and there were no significant treatment interactions (F1,104 = 0.465, P
Leaves of AHM present mesquite were statistically higher in leaf N%, Mg, and Fe concentrations, however leaf P and K concentrations had no treatment effects (Table 3.1).

3.4.2.2. Long-term effect of AHM removal on mesquite reproductive effort

Prior to experimental removal of AHM, flower (AHM Present: 236.17 ± 27.18; AHM Removed: 188.42 ± 21.32) and fruit quantities (AHM Present 338.50 ± 38.59; AHM Removed 328.08 ± 31.17) did not differ in AHM present and AHM removed trees (Table 3.2). Following the establishment of the treatments, the repeated measures GLMM comparing mesquite hosting AHM and AHM removed mesquite indicated that the removal of AHM had significant negative effects on flower quantities (repeated measures GLMM: Wald $x^2 = 11.8, P < 0.001$) but not on fruits (repeated measures GLMM: Wald $x^2 = 0.38, P > 0.05$) and there were significant year effects on flower (repeated measures GLMM: Wald $x^2 = 13.01, P < 0.001$) and fruit quantities (repeated measures GLMM: Wald $x^2 = 21.5, P < 0.0001$). Cattle exclusion had no effect on flower or fruit production and there were no significant interactions with AHM removal, cattle exclusion, and sampling year (Table 3.3). Over the duration of the experiment, mean flower abundance from 2013-2016 was significantly lower in AHM removed mesquite (280.71 ± 16.51) than AHM present mesquite (373.19 ± 20.20; Fig. 3.6a). However, there were no differences in fruit abundance between AHM present (193.85 ± 18.68) and AHM removed mesquite (166.27 ± 15.16; Fig. 3.6b).

Comparisons among AHM present, AHM removed, and AHM naturally absent treatments from 2014-2016 (excluding all fenced individuals from the analysis treatments), revealed that flower abundance was significantly negatively affected by AHM removal (repeated measures GLMM: Wald $x^2 = 9.85, P < 0.01$). Years differed in
flower (repeated measures GLMM: Wald $x^2 = 9.72, P < 0.01$) and fruit quantities (repeated measures GLMM: Wald $x^2 = 27.64, P < 0.0001$), but no treatment by year interaction was detected (Table 3.4). Flower abundance in AHM present mesquite ($400.75 \pm 36.22$) was significantly greater than AHM removed mesquite ($301.72 \pm 29.22$) and mesquite naturally lacking AHM ($262.31 \pm 25.88$; Fig. 3.7a) Fruit abundances were found to not be significantly different among the treatments but varied significantly from 2014 – 2016 as fruit abundance precipitously decreased over the duration of the study (Fig. 3.7b).

3.5. Discussion

Positive feedback loops have been documented in ant-plant mutualisms (Moreira et al. 2012, Pringle 2014, Prior et al. 2014, Prior and Palmer 2018), yet potential of positive feedback loops involving AHM and their host plants is poorly understood. We document here the potential feedback loop between C. sayi and V. segmentata and their host plant, mesquite. The results from this study demonstrate that (1) host quality predicates the presence of AHM as AHM were only present on high quality mesquite, (2) the presence of AHM did not negatively affect mesquite quality, and (3) over the duration of the study mesquite reproductive effort decreased following the removal of AHM.

The importance of host plant quality in the driving establishment and maintenance of AHM has been well documented (Strauss 1987, Silva and Uchida 2000, Cisneros and Godfrey 2001, Stadler et al. 2002, Gove and Rico-Gray 2006, Morales and Beal 2006). For example, honeydew producing hemipterans selected Artemisia ludoviciana enriched with fertilizer over non-enriched plants. In addition, hemipterans on enriched A. ludoviciana experienced increased fecundity, faster development, and attracted higher number of tending ants that were more active (Strauss 1987). Also, populations of aphids
colonizing *Tanacetum vulgare* grown in high nutrient soils experienced higher intrinsic rate of population growth than aphid populations on lower quality *T. vulgare* (Stadler et al. 2002). Although these findings are based on artificially enriched host plants, plants hosting AHM in untreated soil were also found to be of higher quality than plants not hosting AHM (Morales and Beal 2006). At WWR, the prevalence of AHM on mesquite corresponded to mesquite quality. Specifically, mesquite hosting AHM had higher concentrations of macro and micronutrients important for hemipteran growth and reproduction, and lower levels of secondary chemical compounds detrimental to hemipteran development and reproduction. In addition, high quality plants may not only be nutritionally beneficial to hemipterans but also important in attracting, establishing, and maintaining their partnership with ants. The solicitation of ant bodyguards is not solely dependent on the production of honeydew but the quality of honeydew (Cushman 1991, Bronstein 1994, Stadler et al. 2002, Reithel and Billick 2006, Morales 2011) which correlates with plant quality (Völkl et al. 1999, Fischer et al. 2005). Thus, the establishment and maintenance of AHM may not be possible on low quality host due to poor quality honeydew (Cushman 1991, Morales and Beal 2006). This may explain why there were no AHM populations on low quality mesquite.

Though AHM were found on higher quality mesquite, their presence did not negatively affect mesquite quality. Compared to AHM removed and AHM absent mesquite, mesquite hosting AHM recorded higher concentrations of %N, Fe and Mg, that are critical elemental nutrients required for photosynthesis, growth, and flower production. In addition, mesquite hosting AHM to have significantly lower concentrations of condensed tannin, a secondary plant defense compound to thwart
herbivory. The findings from this study corroborate similar studies that have found the presence of AHM do not illicit an increase plant secondary defense compounds such as monoterpenes (Mooney 2007), total phenolics and non-volatile resin (Moreira et al. 2012). Therefore, should the presence of AHM be detrimental, we would expect to see decreases in macronutrients critical to host plant development and increases in secondary defense compounds. Increases in secondary defense compounds and reduced availability of important nutrients would then lead to the loss of AHM (Cushman 1991, Denno et al. 1995).

The beneficial effects of AHM on their host plant reproductive effort have been demonstrated (Ito and Higashi 1991, Del-Claro and Oliveira 2000, Styrsky and Eubanks 2010). However, virtually all these studies measure reproductive effort for only a single season and do not account for temporal variation in reproductive effort. Here, the removal of AHM had a significantly negative long-term effect on flower abundance. It is likely that mesquite flowers are receiving indirect protection from ants as they tend hemipterans populations aggregated at the base of mesquite flowers and fruits. Therefore, V. segmentata aggregations feeding below flowers potentially allowed for larger quantities of flowers to survive due to a reduction of arthropod herbivores (Nasseri 2018). The reduction of herbivores around flowers and fruits by C. sayi tending V. segmentata may explain the higher flower and fruit production on mesquite hosting AHM in 2012 over trees where only C. sayi was present.

Despite the strong effects of AHM presence on flower quantities, fruit production did not differ among treatments over the duration of the study. Fruit production in 2015 and 2016 was ca. 84% lower than in the previous two years across all trees. The summers
of 2015 and 2016 were unusually wet, causing the loss of ground nesting bee populations.

Ground nesting bees are highly susceptible to flooding and require several generations to rebound (Fellendorf et al. 2004) and, in 2015, surveys of pollinators in cotton farms surrounding the WWR found bee abundances and richness virtually non-existent compared to prior sampling years (Cusser et al. in review). Their loss may explain why mesquite failed to set fruit regardless of high flower production.

3.5.1. Conclusion

Our study is one of the first to provide empirical evidence from a long-term manipulative experiment on a potential positive feedback loop between host plant quality and the protective benefits incurred by AHM. Similar to feedback loops reported in ant-plant mutualisms in which ants defend plants from herbivores and competitors and thus allow plants increased investment into food rewards and/or domatia (Frederickson 2009, Pringle 2014). We observed very similar feedbacks in relation to tending ants, honeydew producing hemipterans and their host plants. The caveat being, defensive service by ants is mediated by honeydew producing hemipteran feeding which is dependent on their host. Thus, the feedback loop is only possible if (1) AHM are dependent on their host plant quality, (2) the presence of AHM are not detrimental to their host plant over the long-term, and (3) presence of AHM have strong, positive long-term effect on their host plant reproductive effort. Given the positive feedback loop recorded in this study, the strength of these interactions may have cascading community level impacts effects beyond AHM and their host plant (Nasseri et al 2018). Therefore, we need to incorporate more long-term studies focusing on the potential community level consequences of either losing or gaining these strong interactions.
3.6. Reference


Lach, L. 2007. A Mutualism with a Native Membracid Facilitates Pollinator Displacement by Argentine Ants Author (s): Lori Lach Published by: Ecological Society of America content in a trusted digital archive. We use information technology and tools to increase produ 88:1994–2004.


Rosumek, F. B., F. A. O. Silveira, F. De S. Neves, N. P. Newton, L. Diniz, Y. Oki, F.


Table 3.1. MANOVA results: Mesquite leaf macronutrient concentrations (percent foliar nitrogen (%N), iron (Fe) in part per million (ppm), magnesium (Mg ppm), potassium (K ppm), phosphorous (P ppm)) in 2015 across treatments (ant-hemipteran mutualism removal (AHM) and cattle enclosures (Fence)). For the MANOVA, Pillai’s trace was used as the test statistic. Univariate ANOVA used to test the effect of AHM, fence and AHM by fence interactions on individual macronutrients. F-values were used as the test statistic. Multiple comparisons on univariate ANOVA were done with Tukey Kramer post hoc test. Different letters (a,b) indicate significance at P < 0.05. * P < 0.05, ** P < 0.01, ***P < 0.001, n.s. = non-significant (P > 0.05). S.E. = mean standard error.

Table 3.2. Generalized linear model testing for differences in flower and fruit production of mesquite in 2013 prior to the establishment of ant-hemipteran mutualism (AHM) removal and cattle exclusion (Fence) treatments.

Table 3.3. Generalized linear mixed models testing for the effect of ant-hemipteran mutualism (AHM) removal and cattle exclusion (Fence) on mesquite flower and fruit production from 2013-2016.

Table 3.4. Generalized linear mixed models testing for the effect of ant-hemipteran mutualism (AHM) removal on mesquite flower and fruit production from 2014-2016.
Table 3.1. MANOVA results: Mesquite leaf macronutrient concentrations (percent foliar nitrogen (%N), iron (Fe) in part per million (ppm), magnesium (Mg ppm), potassium (K ppm), phosphorous (P ppm)) in 2015 across treatments (ant-hemipteran mutualism removal (AHM) and cattle enclosures (Fence)). For the MANOVA, Pillai’s trace was used as the test statistic. Univariate ANOVA used to test the effect of AHM, fence and AHM by fence interactions on individual macronutrients. F-values were used as the test statistic. Multiple comparisons on univariate ANOVA were done with Tukey Kramer post hoc test. Different letters (a,b) indicate significance at $P < 0.05$, $* P < 0.05$, $** P < 0.01$, $*** P < 0.001$, n.s. = non-significant ($P > 0.05$). S.E. = mean standard error.

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<td>0.04</td>
<td>0.53n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Removed</td>
<td>15,508.6±386</td>
<td></td>
<td>0.18</td>
<td>1.87n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Absent</td>
<td>13,973.7±643</td>
<td></td>
<td>0.04</td>
<td>0.53n.s.</td>
</tr>
<tr>
<td></td>
<td>Fence</td>
<td>Yes</td>
<td>15,074.6±275</td>
<td>1,104</td>
<td>0.12</td>
<td>2.35n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>15,421.8±386</td>
<td></td>
<td>0.04</td>
<td>0.53n.s.</td>
</tr>
<tr>
<td>P ppm</td>
<td>AHM</td>
<td>Present</td>
<td>2033.1±1.8</td>
<td>1,104</td>
<td>0.002</td>
<td>0.04n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Removed</td>
<td>1958.7±76.7</td>
<td></td>
<td>0.12</td>
<td>1.87n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Absent</td>
<td>1757.4±82.8</td>
<td></td>
<td>0.002</td>
<td>0.04n.s.</td>
</tr>
<tr>
<td></td>
<td>Fence</td>
<td>Yes</td>
<td>1979.6±65.4</td>
<td>1,104</td>
<td>0.12</td>
<td>1.87n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>1957.6±68.4</td>
<td></td>
<td>0.002</td>
<td>0.04n.s.</td>
</tr>
<tr>
<td></td>
<td>AHM*Fence</td>
<td>Present</td>
<td>1,104</td>
<td>0.005</td>
<td>0.10n.s.</td>
<td></td>
</tr>
</tbody>
</table>

†marginal significance at $P = 0.06$
Table 3.2. Generalized linear model testing for differences in flower and fruit production of mesquite in 2013 prior to the establishment of ant-hemipteran mutualism (AHM) removal and cattle exclusion (Fence) treatments.

<table>
<thead>
<tr>
<th>2013 Fixed Effect</th>
<th>Flower Wald $x^2$</th>
<th>DF</th>
<th>P value</th>
<th>Fruit Wald $x^2$</th>
<th>DF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHM Presence</td>
<td>1.62</td>
<td>1</td>
<td>&gt; 0.05</td>
<td>0.03</td>
<td>1</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Fence Presence</td>
<td>0.27</td>
<td>1</td>
<td>&gt; 0.05</td>
<td>0.03</td>
<td>1</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>AHM*Fence</td>
<td>0.28</td>
<td>1</td>
<td>&gt; 0.05</td>
<td>1.5</td>
<td>1</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>
Table 3.3. Generalized linear mixed models testing for the effect of ant-hemipteran mutualism (AHM) removal and cattle exclusion (Fence) on mesquite flower and fruit production from 2013-2016.

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>2013-2016</th>
<th>Flower</th>
<th></th>
<th>Fruit</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wald $x^2$</td>
<td>DF</td>
<td>$P$ value</td>
<td>Wald $x^2$</td>
<td>DF</td>
</tr>
<tr>
<td>AHM Presence</td>
<td>11.84</td>
<td>1</td>
<td>$&lt; 0.001$</td>
<td>0.38</td>
<td>1</td>
</tr>
<tr>
<td>Fence Presence</td>
<td>3.13</td>
<td>1</td>
<td>$&gt; 0.05$</td>
<td>0.04</td>
<td>1</td>
</tr>
<tr>
<td>Year</td>
<td>13.01</td>
<td>1</td>
<td>$&lt; 0.001$</td>
<td>21.55</td>
<td>1</td>
</tr>
<tr>
<td>AHM*Fence</td>
<td>0.07</td>
<td>1</td>
<td>$&gt; 0.05$</td>
<td>3.74</td>
<td>1</td>
</tr>
<tr>
<td>AHM*Year</td>
<td>0.09</td>
<td>1</td>
<td>$&gt; 0.05$</td>
<td>0.45</td>
<td>1</td>
</tr>
<tr>
<td>Fence*Year</td>
<td>0.41</td>
<td>1</td>
<td>$&gt; 0.05$</td>
<td>0.05</td>
<td>1</td>
</tr>
<tr>
<td>AHM<em>Fence</em>Year</td>
<td>0.04</td>
<td>1</td>
<td>$&gt; 0.05$</td>
<td>1.07</td>
<td>1</td>
</tr>
</tbody>
</table>

†AHM absent trees removed from the analysis
Table 3.4. Generalized linear mixed models testing for the effect of ant-hemipteran mutualism (AHM) removal on mesquite flower and fruit production from 2014-2016.

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>2014-2016</th>
<th>Flower</th>
<th>Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wald x²</td>
<td>d.f.</td>
<td>P value</td>
</tr>
<tr>
<td>AHM Presence</td>
<td>9.85</td>
<td>2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Year</td>
<td>9.72</td>
<td>1</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>AHM*Year</td>
<td>2.36</td>
<td>2</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

†Fenced trees removed from the analysis
3.8. Figure Legend

Figure 3.1. Condensed tannin (%LE) production in 2012 from honey mesquite hosting either ant-hemipteran mutualisms (AHM; Present), mesquite containing only ants (Ants Only), or mesquite containing no ants (Absent). Difference between groups were determined using the Tukey method post hoc test. Dissimilar letters indicate significant differences at \( P < 0.05 \).

Figure 3.2. Relationship between mesquite condensed tannin (%LE) production and cattle dung densities in 2012. There was a significant interaction term between AHM presence and dung densities. Each panel shows the relationship of CT in relation to cattle dung densities depending on the presence of AHM and lack of ants.

Figure 3.3. Flower production in 2012 from honey mesquite hosting either ant-hemipteran mutualisms (AHM; Present), ants only, or mesquite containing no ants (Absent). Difference between groups were determined using the Tukey method post hoc test. Dissimilar letters indicate significant differences at \( P < 0.05 \).

Figure 3.4. Fruit production in 2012 from honey mesquite hosting either ant-hemipteran mutualisms (AHM; Present), mesquite containing only ants (Ants Only), or mesquite containing no ants (Absent). Difference between groups were determined using the Tukey method post hoc test. Dissimilar letters indicate significant differences at \( P < 0.05 \).

Figure 3.5a. Linear relationship between mesquite flower production and cattle dung densities in 2012.

Figure 3.5b. Linear relationship between mesquite fruit production and cattle dung densities in 2012.

Figure 3.6a. A. Mean (± S.E.) abundance of mesquite flowers from 2013-2016 in which the presence of ant-hemipteran mutualisms (AHM) was manipulated on honey mesquite. (B) Flower abundance partitioned by AHM and fencing treatment. Present = ants not removed; Removed = ants experimentally removed.

Figure 3.6b. A. Mean (± S.E.) abundance of fruit production from 2013-2016 in which the presence of ant-hemipteran mutualisms (AHM) was manipulated on honey mesquite. Present = ants not removed; Removed = ants experimentally removed. (B) Flower abundance partitioned by AHM and fencing treatment. Present = ants not removed; Removed = ants experimentally removed.

Figure 3.7a. Mean (± S.E.) abundance of flower production from 2014-2016 in which the presence of ant-hemipteran mutualisms (AHM) was manipulated on honey mesquite. Present = ants not removed; Removed = ants experimentally removed; Absent = ants naturally absent.
†Fenced trees removed from this analysis.
††See Table 3.3 for statistics.

Figure 3.7b. Mean (± S.E.) abundance of fruit production from 2014-2016 in which the presence of ant-hemipteran mutualisms (AHM) was manipulated on honey mesquite.
Present = ants not removed; Removed = ants experimentally removed; Absent = ants naturally absent.
†Fenced trees removed from this analysis.
††See Table 3.3 for statistics.
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Figure 3.2. Relationship between mesquite condensed tannin (%LE) production and cattle dung densities in 2012. There was a significant interaction term between AHM presence and dung densities. Each panel shows the relationship of CT in relation to cattle dung densities depending on the presence of AHM and lack of ants.
Figure 3.3. Flower production in 2012 from honey mesquite hosting either ant-hemipteran mutualisms (AHM; Present), ants only, or mesquite containing no ants (Absent). Difference between groups were determined using the Tukey method post hoc test. Dissimilar letters indicate significant differences at P < 0.05.
Figure 3.4. Fruit production in 2012 from honey mesquite hosting either ant-hemipteran mutualisms (AHM; Present), mesquite containing only ants (Ants Only), or mesquite containing no ants (Absent). Difference between groups were determined using the Tukey method post hoc test. Dissimilar letters indicate significant differences at P < 0.05.
Figure 3.5a. Linear relationship between mesquite flower production and cattle dung densities in 2012.

Figure 3a. Linear relationship between mesquite flower production and cattle dung densities in 2012.
Figure 3b. Linear relationship between mesquite fruit production and cattle dung densities in 2012.

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†Fenced trees removed from this analysis.
†††See Table 3 for statistics.
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†Fenced trees removed from this analysis.
†††See Table 3 for statistics.
CHAPTER 4: THE EFFECT OF ANT-HEMIPTERAN MUTUALISMS ON THE INVERTEBRATE COMMUNITY ON HONEY MESQUITE (PROSOPIS GLANDULOSA)

4.1. Abstract:
The importance of species interactions in driving patterns of invertebrate abundance and diversity have been a central focus for ecologists. Ants engaged in mutualistic interactions with other invertebrates may affect arthropod communities by acting defending their mutualist partners from enemies and competitors. However, the community level effects of ant-hemipteran mutualisms (AHM) are still poorly understood. We hypothesized that a mutualism between carpenter ants, Camponotus sayi, and a honeydew producing hemipteran, Vanduzea segmentata, act as important drivers in how (1) the arthropod community and (2) functional arthropod feeding guilds are structured. In 2013, we established a three-year press experiment in which we removed ants on honey mesquite, Prosopis glandulosa Torr., and measured changes in total arthropod abundance, family richness and diversity, β diversity, and turnover in community composition from one year to the next. The removal of AHM had no effect on total family richness, but AHM removed treatments were significantly more diverse than AHM present mesquite. The removal of AHM resulted in significantly lower β diversity on trees from which ants were removed (P <0.05). The turnover rate in both treatments were similar and driven by the replacement of families and not by the addition or loss of families. Arthropod feeding guilds also shifted in response to AHM (P <0.001). Chewing herbivores and predators increased post-removal, while pollinators decreased. The community level effects of AHM removal were temporally variable. These results support the role of mutualistic interactions affecting the structure of arthropod communities. The temporal variability found emphasizes the importance of using multi-seasonal, community level studies to gain a complete understanding of the ecological importance of AHM.
4.2. Introduction


Only recently, have AHM started to be recognized as “keystone interactions” capable of altering, driving and maintaining communities through the aggressive and defensive behavior of tending ants (Eubanks and Styrsky 2006, Styrsky and Eubanks 2007, Clark and Singer 2018, Prior and Palmer 2018). However, studies investigating the role of AHM on arthropod communities have generally focused on AHM effects on individual species of interests or small assemblages of specific species. To date, only a few studies have measured the effect AHM have on the entire arthropod community.
(Fowler and MacGavin 1985; Wimp and Whitham 2001; Kaplan and Eubanks 2005; Lach 2007; Mooney 2007; de Freitas and Rossi 2015) with inconsistent results. For example, two of the studies found that the removal of AHM resulted in significant increases in arthropod abundance and richness (Fowler and MacGarvin 1985, Wimp and Whitham 2001). Another two found that the removal of AHM found no effect on arthropod abundance and richness (Lach 2007, de Freitas and Rossi 2015). The inconsistencies with these may be a result of using abundances and species richness as metrics in assessing the community level effects of AHM. Species richness and abundance measures are not comparable across studies due to the correlative biases of sampling effort (i.e. number of sites or sampled).

Therefore, to gain a deeper and more long-term understanding of the effects of AHM on structuring communities, we established a multiyear study investigating the effect of long-term removal of AHM on the arthropod community of honey mesquite (Prosopis glandulosa). Specifically, we tested the hypotheses that (1) arthropod communities and (2) arthropod feeding guilds on mesquite hosting AHM differ in the presence of AHM. To address our first hypothesis, we compared community level effects of AHM removal utilizing species richness and diversity (α diversity). We also compared beta (β) diversity in terms of the compositional change in the arthropod community due to AHM removal and as the rate arthropod communities changed across years. Finally, we measured how the composition of ecologically relevant arthropod feeding guilds were affected by the removal of AHM.
4.3. Methods

4.3.1. Study Site
Our work was conducted at the Rob and Bessie Welder Wildlife Refuge (WWR) in San Patricio County, TX (28°06’31.78”N, 97°23’56.15’’W). WWR is part of the Gulf Coastal Plains region and contains nine unique plant communities (Glasscock et al. 2005). Our study was conducted within the “mesquite-mixedgrass” plant community. This ecotype is represented by primarily black Victorian clay hosting a grassland savanna community and dominated by honey mesquite (*Prosopis glandulosa*). WWR is primarily xeric with a bimodal peak in rainfall occurring in September and May-June (Glasscock et al. 2005).

4.3.2. Host Plant: Mesquite
Mesquite is a nitrogen-fixing legume native to Central America and is one of the top 100 of the world’s worst invasive alien species (Lowe et al. 2000). Mesquite is a significant problem in the Panhandle of Texas into Mexico and Arizona. It forms dense, impenetrable thickets, out-competes native shrubs and grasses, and thus degrades natural habitats. In southern Texas, it has overtaken cattle pastures as well as replaced native live oaks as the dominant hard wood (Fulbright et al. 1990, Griffith et al. 2004). Mesquite flowers from May – September with peak production in June and July (McGrath et al. 2009). Its inflorescences are showy yellow racemes that produce large quantities of pollen and are highly attractive to floral visitors. Its fruits are an important source of food during the summer months for a wide variety of organisms (Vines 1960).

4.3.3. Ant-Hemipteran Mutualism
At WWR, the ant-hemipteran mutualism is comprised of *Camponotus sayi* tending *Vanduzea segmentata*. We identified four other genera of ants (*Camponotus sayi, Crematogaster* sp., *Dorymyrmex* sp., and *Solenopsis* spp.) on mesquite, but only ever
found *C. sayi* tending *Vanduzea segmentata*. *Camponotus sayi* were generally found to be nesting at the base of mesquite trunks in fissures beneath the bark or in dead branches. Workers would move onto the branches and canopy of mesquite to harvest honeydew produced from *V. segmentata* as well as to forage for invertebrate prey.

*Vanduzea segmentata* are native to Central America, however, very little is known of their range expansion into North America and their life history. Based on personal observations from N. Nasseri, *V. segmentata* aggregate at the base of flowering racemes or seed pods for the duration of the mesquite reproductive season. Their emergence appears to be strongly tied to the flowering phenology of mesquite.

### 4.3.4. AHM removal

To examine the effect of AHM on the invertebrate community, in May 2013, we selected 100 mesquite trees >2.5m in height (4.13 ± 0.11m) with non-overlapping canopies hosting AHMs. Trees were then randomly assigned to one of two treatments: AHM removed (N = 50) or controls (AHM present, N = 50). In May 2014, we added an additional 30 mesquite trees that naturally did not host AHMs (“Absent”) to determine if the invertebrate community sampled on mesquite that naturally did not host AHM differed from mesquite hosting AHMs (“Present”) and mesquite with AHMs experimentally removed (“Removed”). We established and maintained a four year (2013-2016) ant removal press experiment (Yodzis 1988). Ants were removed with commercially available granular ant poison baits. We installed three bait stations (15 cm long, 6.35 mm wide polyvinyl chloride (PVC) pipe) per tree. Along the length of the PVC, 2 mm holes were drilled to allow access by ants and reduce contact by non-target species (Klimes et al. 2011; Nasseri 2018). We filled each station with commercially available granular ant poison baits. We maintained the treatments by recharging stations
with fresh bait every six months to prevent ants from recolonizing AHM removed trees. In each year we monitored all trees for AHM presence and absence monthly from April to August to ensure treatment effectiveness (Nasseri 2018). To control for possible effects the presence of ant bait stations may have on non-target insects, we randomly selected 15 of the AHM absent trees to receive charged bait stations like our ant removal treatments and the other half remained as un-baited controls. We also added an additional 15 mesquite trees hosting AHM to serve as an un-baited AHM present control.

4.3.5. Invertebrate collection
To assess how tending ants affected the arthropod community, we sampled the natural, unmanipulated arthropod community before the establishment of the ant removal treatments (June 2013), and six weeks after ants were removed (August 2013). Using a modified leaf mulcher (Ryobi 2cc leaf blower/mulcher) each tree was vacuum sampled for six minutes. To reduce temporal affects we restricted sampling from 8:00h – 11:00h. Sampling was conducted from 1m off the ground to 3m in height with a right to left sweep while walking around the tree. Sampling was not specific to branches that contained AHM but random sweeping of the entire area to maximize captures. The contents of the vacuum were placed in zip-lock bags with a 4x4cm sponge saturated with 100% acetone and transported back to the research station. Sampled arthropods were then placed in labeled 1dram vials containing 70% EtOH after sorting out from the debris. Samples were then transported back to the University of Vermont for identification and quantification. We identified specimens to Family level and quantified taxonomic richness and abundance for all treatment trees.
4.3.6. Statistical Approach

4.3.6.1. Effect of AHM on arthropod community composition

To determine the long-term effect of AHM removal on the arthropod community, we estimated species richness (Hill number q = 0) and α diversity (q = 1) for each tree based on abundance data and created rarefaction and extrapolation (R/E) curves using the R package “iNEXT” (Hsieh et al. 2016). Following the recommendations of Chao et al. (2014), extrapolations were conducted to no more than twice the reference sample size and R/E curves were plotted with 95% confidence intervals calculated using a bootstrap method with 5000 replications. Confidence intervals not overlapping were considered significant at P > 0.05 (Chao et al. 2014).

To measure both $\beta_{\text{VAR}}$ and $\beta_{\text{TURN}}$, we used the function “betapart.core” in the R package betapart (Baselga and Orme 2012) to create dissimilarity matrices on incidence data (Sørensen dissimilarity index). $\beta_{\text{VAR}}$ was calculated using the function beta.pair to compute a pairwise between-site value ($\beta_{\text{VAR}}$). We used the function “betadisper” in Vegan to run 1000 permutations on the distance matrices (homogeneity of multivariate dispersions) to test if the average distance from the group centroid among sampling sites were significantly different. This is a recommend method to assess $\beta_{\text{VAR}}$ diversity (Anderson et al. 2006). We used the function “beta.temp” to compute Sørensen dissimilarity values between matched sites from two different years. This function then returns values for $\beta_{\text{TURN}}$, $\beta_{\text{SIM}}$, and $\beta_{\text{NES}}$.

4.3.6.2. Effect of AHM on Arthropod Functional Guilds

Our second objective was to determine the effect of AHM on functional arthropod feeding guilds – which Stroud et al. (2015) describes as “a group of functionally similar species that exploit the same class of resources”. Based on this definition, we classified arthropods according to six feeding guilds: “non-tending ants”, “chewing herbivores”, 
“parasitoids”, “pollinators”, “parasitoids”, and “sucking herbivores”. We grouped arthropods into guilds based on the morphology of their mouth parts and feeding behaviors described from the literature, insect identification guides, and consultation with taxonomic specialists. We then compared guild abundances between our treatments using the `manyglm` function in the R package “mvabund” (Wang et al. 2012). A repeated measure analysis was achieved by blocking “tree id” within the model (Wang et al. 2012). We present univariate results from `manyglm` as unadjusted p-values due to an unacceptable reduction in power when correcting for more than five variables resulting in too conservative test results (Moran 2003).

4.4. Results
4.4.1. Mesquite arthropod community
In three years of vacuum sampling mesquite at WWR, we collected a total of 36,275 individuals in 80 families. *C. sayi* (2,880 individuals) and *V. segmentata* (2,790 individuals) in addition to abundances from six families (Braconidae, Chrysomelidae, Eurytomidae, Hybotidae, Miturgidae, and Pteromalidae) containing over 1,000 individuals in each family which contributed to 70.7% of the total abundance. Conversely, there were 31 families with less than 100 individuals sampled (Fig. 4.1). Overall, arthropod abundance and family richness on mesquite remained stable over the duration of the study (Table 4.1). The family accumulation (Fig. 4.2a) and the R/E family richness curve (Fig. 4.2b) indicated that we sampled ca. 99% of the estimated arthropod families on mesquite.

4.4.1.1. Efficacy of AHM removal
The mutualism between *V. segmentata* and *C. sayi* exhibited strong host fidelity with most of the mesquite maintaining their status as AHM hosts. Prior to the application of bait stations, the abundance of *C. sayi* (16.2 ± 2.3/tree) and *V. segmentata* (18.0 ±
2.6/tree) in AHM present trees did not differ from AHM removed trees (C. sayi: 16.6 ± 2.2/tree, Wald $x^2 = 0.02$, P >0.05; V. segmentata: 18.0 ± 2.6, Wald $x^2 = 0.69$, P >0.05, respectively; Fig. 4.3). At six-weeks post ant removal, C. sayi and V. segmentata were virtually extirpated from all AHM removal trees (C. sayi: 0.22 ± 0.1/tree, V. segmentata: 0.56 ± 0.2/tree) and significantly less abundant than the control AHM present trees (C. sayi: 12.1 ± 1.4/tree, Wald $x^2 = 202.4$, P <0.0001; V. segmentata: 10.7 ± 2.4/tree individuals/tree, Wald $x^2 = 86.9$, P <0.0001). Camponotus sayi was found to be significantly positively correlated to V. segmentata over the duration of the study as the removal of C. sayi resulted in the loss of V. segmentata ($F_{1,370} = 384.7$, $R^2 = 0.51$, P <0.0001; Fig. 4.4). In addition, the bait stations successfully suppressed the recolonization of ant removal trees by AHMs for the duration of the study (Fig. 4.3a,b). We found no evidence that the ant bait stations negatively affected non-target species. The sham bait stations (empty PVC pipes) on mesquite hosting AHM and mesquite naturally lacking AHM did not differ in family richness (Hill’s q = 0; Fig. 4.5 and 4.6, respectively) or α diversity (Hill’s q = 1; Fig. 4.5 and 4.6). Furthermore, bait stations did not affect guild assemblage (AHM present: LRT$^{1,58} = 3.76$, P >0.05; AHM removed: LRT$^{1,26} = 2.22$, P >0.05; Table 4.2).

4.4.1.2. Effect of AHM on invertebrate richness and diversity

Prior to the removal of AHM in 2013, family richness on AHM present trees was 71 ± 10.2 compared to 80 ± 16.5 in mesquite designated for AHM removal (Table 4.1). The corresponding family richness and α diversity R/E curves constructed did not differ based on overlapping 95% CL (Fig. 4.7, respectively). Likewise, the community composition in AHM present and removed mesquite did not differ (homogeneity of multivariate dispersion: permuted $F_{1,96} = 0.421$, P >0.05).
The compositional difference in family assemblages from 2013 to 2014 was 0.475 ± 0.02 in mesquite hosting AHM and 0.464 ± 0.01 in AHM removed mesquite. Family replacement ($\beta_{SIM}$) drove the compositional shift of family turnover in AHM present and AHM removed trees (AHM present: $\beta_{SIM} = 0.380 \pm 0.02$; AHM removed: $\beta_{SIM} = 0.390 \pm 0.01$) rather than the loss or addition of new families (AHM Present: $\beta_{NES} = 0.095 \pm 0.01$; AHM Removed: $\beta_{NES} = 0.075 \pm 0.01$). In 2014, family richness of AHM present (70.4 ± 5.9) and AHM removed (68.6 ± 4.2) trees were comparable; and the constructed R/E curves for family richness did not differentiate (overlapping 95% CL; Fig. 4.8). Alpha diversity R/E curves for AHM present mesquite were marginally lower compared to AHM removed mesquite (Fig. 4.8). As such, the variance in the invertebrate assemblages in AHM present mesquite was significantly higher than AHM removed trees (homogeneity of multivariate dispersion: permuted $F_{1,116} = 3.58$, $P < 0.05$).

In 2015 the rate of family turnover of the invertebrate assemblage from 2014 in AHM present mesquite ($\beta_{TURN} = 0.481 \pm 0.02$) was not statistically different from that of the AHM removed mesquite ($\beta_{TURN} = 0.481 \pm 0.01$). In removal treatments and AHM present mesquite, 80-83% of the total compositional change from 2014 to 2015 was a result of the replacement ($\beta_{SIM}$), rather than the addition ($\beta_{NES}$) of new families. Family richness was highly variable among trees and did not differ between treatments (AHM present: 83.0 ± 20.2, AHM removed: 62.7 ± 10.3; Fig. 4.9). Alpha diversity on mesquite hosting AHM was significantly lower than AHM removed trees (Fig. 4.9). However, there was no difference in the variation of invertebrate assemblages due to AHM removal (homogeneity of multivariate dispersion: $F_{1,123} = 1.15$, $P > 0.05$).
4.4.1.3. Effect of AHM on Arthropod Functional Guilds

Guild composition did not differ between AHM present and AHM removal trees prior to the removal of AHM in 2013 (LRT\(1,96\) = 2.09, P >0.05, Fig. 4.10), but significant effects of AHM removal on guild assemblages were measured from 2014-2015 (LRT\(1,173\) = 11.83, P >0.0001; Table 4.3). AHM present mesquite had lower abundance of chewing insects (AHM present = 15.9 ± 2.3; AHM removed = 21.2 ± 1.8, unadjusted P <0.05) and insect predators (AHM present = 19.3 ± 1.5; AHM removed = 15.4 ± 1.1, unadjusted P <0.05), and higher abundance of pollinators (AHM present = 5.2 ± 0.9; AHM removed = 3.2 ± 0.5, unadjusted P <0.05) and V. segmentata (AHM present = 16.1 ± 2.5; AHM removed = 0.78 ± 0.2, unadjusted P <0.05; Fig. 4.11). In 2015, AHM present mesquite contained significantly higher abundances of parasitoids (AHM present = 86.2 ± 14.5; AHM removed = 53.8 ± 8.6, unadjusted P <0.05) and V. segmentata (AHM present = 7.7 ± 1.5; AHM removed = 0.51 ± 0.32, unadjusted P >0.05; Fig. 4.12).

4.5. Discussion

Ant–hemipteran mutualisms have been described as “keystone interactions” as they can be major drivers in structuring communities through direct and indirect effects (O’Dowd et al. 2003, Styrsky and Eubanks 2007, Grinath et al. 2012). However, the direct and indirect effects of AHMs on communities have not actually been measured at the community level. When investigating community level effects of AHM, studies have primarily centered on individual species of interest, or small, specified assemblages of hemipteran predators (Kaplan and Eubanks 2005), parasitoids (Grover et al. 2008), non-honeydew producing herbivores (Clark and Singer 2018), and pollinators (Lach 2007; Ibarra-Isassi and Oliveira 2018) within a single season. Comparing the change in community structure over time and the variations in arthropod assemblages after the loss
of AHM may indicate the spatial and temporal importance of AHMs. We conducted a multiyear, AHM press removal study that allowed for the comparison of the arboreal arthropod family composition and guild assemblages from mesquite hosting AHM and AHM removed mesquite over three years. Our study provides one of the first multi-year comparisons of the effects of the loss of AHM on arthropod richness, α diversity, variation in β diversity, temporal turnover, and the compositional shift in arthropod feeding guilds. Thus, these findings expand our current understanding of the role AHM have as keystone interactions and accentuates the importance of broadening our scope to multi-seasonal, community level studies on the ecological importance of AHM.

Mesquite hosted abundant and diverse arthropod communities that did not differ prior to the removal of AHM. Following the removal of AHM, the overall turnover rate within the treatments was driven by the replacement of families rather than the loss or addition of new families. The replacement of families rather than the loss of families indicates that some families can tolerate or avoid tending ants whereas others are potentially driven away by ants to be replaced by other individuals. Thus, although α diversity is changing, species richness is not. This may be why some studies do not find that the removal of AHM significantly increase species abundance and richness (Lach 2007, de Freitas and Rossi 2015). Based on α diversity R/E curves, we found the presence of AHMs decreased α diversity compared to AHM removed mesquite over the duration of the study. This indicates that the arthropod assemblage on AHM removed mesquite was more diverse than AHM present mesquite. Furthermore, there was a significant change in the arthropod community composition due to AHM removal in 2014 but not 2015. The reduction in arthropod diversity and compositional difference in
AHM present mesquite is most likely due to the aggressive behavior of tending ants physically removing arthropods, or arthropods may actively avoid mesquite hosting AHM. We may not have observed any difference in the community composition in 2015 due to the high variation in family richness and reduced populations of *C. sayi* and *V. segmentata* recorded in 2015, both of which may have been driven by historical flooding at WWR in 2015. Based on these results, the general hypotheses that the presence of AHM negatively affects the arthropod community is rejected in terms of richness and abundance but supported in regards α and β diversity. Thus, our study illustrates that incorporating α and β (turnover and variation) diversity measures and multi-season sampling provides a more comprehensive understanding of the community level effects of AHM compared to single season measurements of taxonomic richness.

Prior to the removal of AHM in 2013, the composition of arthropod feeding guilds of mesquite was similar. In 2014, we detected a significant shift in the composition of arthropod feeding guilds after the removal of AHM. Removing AHMs benefitted predators and chewing herbivores, but had no effect on sucking herbivores and parasitoids, and detrimental to pollinators. In 2015, all main effects of AHM removal on feeding guilds disappeared; except parasitoids were found to be negatively affected by AHM removal.

Consistent with the studies measuring the effects of AHM on arthropod guilds and individual species within guilds ((Messina 1981, Morales 2000, Grover et al. 2008, Styrsky and Eubanks 2010), in 2014, we found the presence of AHM in mesquite to be detrimental to herbivore and predator populations. These findings suggest that herbivores and predators are more sensitive to the presence of tending ants, potentially due to
increased chance of encounters as they share the same space and generally compete for the same resources (Kaplan and Eubanks 2005). This may also explain why in our study we find significantly lower numbers of predators in AHM present mesquite as *V. segmentata* is highly vulnerable to predation as indicated by their obligate dependence on the presence of *C. sayi*. Furthermore, conflict over shared space and resources could explain why we recorded significant positive effects of AHM removal on chewing herbivores but not sucking herbivores. The dominant chewing herbivore within the study site was a chrysomelid beetle that depends on mesquite flowers and fruits for food, reproduction, oviposition and larval development as does *V. segmentata*. Thus, *V. segmentata* protected by *C. sayi* can monopolize this crucial resource resulting in reduced populations of the dominant chewing herbivore. Conversely, we see no effect of AHM presence on sucking herbivores as the three most common are xylem feeders. Therefore, sucking herbivores may not compete with the phloem feeding *V. segmentata*; thus they avoid interactions with tending ants. Increases in both herbivores and predators after the removal of AHM also indicates that these two guilds are responding positively to the loss of antagonistic tending ants and perhaps to newly open feeding and breeding sites. Furthermore, predators may doubly benefit due to the increase of greater prey availability with growing herbivore populations (de Freitas and Rossi 2015).

Pollinators decreasing in the absence of AHM was surprising given previous studies that found detrimental effects of AHM on pollinators (Lach 2007). Our result may be an indirect effect of tending ants reducing predator abundances, particularly spiders. For instance, crab spiders (Thomosidae) are perfectly camouflaged ambush hunters that specialize in hunting floral visitors (Suttle 2003). In other studies, butterflies
preferentially select plants with ant activity for foraging and as oviposition sites (Mota and Oliveira 2016, Wynhoff and van Langevelde 2017, Carleial et al. 2018). More work is needed to tease out mechanisms and to describe the full effects of AHM on pollinators.

In 2014, there was no significant effect of AHM removal on parasitoids. However, in 2015, we found significantly more parasitoids on trees hosting AHM. Based on previous studies on specific species of parasitoids, we would expect parasitoids to either not be affected or negatively affected by the presence of AHM. De Freitas and Rossi (2015) found high, albeit nonsignificant, abundances of parasitoids in the presence of AHM; however, they claimed the mutualism was not the cause of the increased abundance. The significantly high abundance of parasitoids in the presence of AHM was potentially an artifact of the extreme weather WWR experienced in 2015. Due to extreme flooding, mesquite flowering was delayed by several weeks; and the emergence and abundance of many species of arthropods that depend on mesquite flower and fruit may have been delayed as well. At the time of sampling, mesquite hosting AHM had the highest quantities of flower (Nasseri 2018) and thus parasitoids may have been utilizing the abundant flowers for nectar. These results again illustrate how community level measurements and multi-seasonal sampling are required to gain a more comprehensive perspective on how AHMs affect other arthropods. Our three-year AHM removal study indicates that the measured effects of AHM removal can be temporally inconsistent and the presence of AHM can have detrimental effects on ecologically important arthropod feeding guilds, biased towards the AHM antagonist.

4.5.1. Conclusion
Several of our results are in accordance with the few studies measuring the effect of AHM on the overall arthropod community (Fowler and MacGarvin 1985, Sakata 1995,

However, differing from those studies, we expanded the temporal scope of our study to measuring the effect of AHM removal over three years. By doing so, we were able to capture the rate of turnover and the mechanism of turnover in communities with and without AHM for the first time. This study demonstrates the ecological importance of mutualisms do not solely rely on a single metric measuring the compositional shift of a small assemblage of species or feeding guild. To gain a comprehensive picture of the ecological consequence of losing strong mutualistic interactions we need to expand from single season, single metric studies to multi-season, community level studies.
4.6. Reference


Lach, L. 2007. A Mutualism with a Native Membracid Facilitates Pollinator Displacement by Argentine Ants Author ( s ): Lori Lach Published by : Ecological Society of America content in a trusted digital archive . We use information technology and tools to increase produ 88:1994–2004.


Messina, F. 1981. Plant Protection as a Consequence of an Ant-Membracid Mutualism :
Interactions on Goldenrod (Solidago Sp.). Ecology 62:1433–1440.


4.7. Table Legend

Table 4.1 Total abundance of arthropods, Camponotus sayi, and Vanduzea segmentata and observed (Obs. Richness) and estimated (Chao1) family richness partitioned by year and AHM treatment.

Table 4.2. Generalized linear model (GLM) results testing the effects of ant-bait stations on arthropod feeding guilds in 2014. We compared arthropod feeding guilds on ant-hemipteran present (AHM) mesquite that contained no bait stations (Control) to those containing empty bait stations (sham treatment). We also compared the effect of poisoned bait stations (Charged) to empty bait stations (sham) on arthropod feeding guilds in mesquite naturally lacking AHM.

Table 4.3. Repeated measure generalized linear model (GLM) results testing the effects of ant-hemipteran mutualisms (AHM) removal on mesquite arthropod feeding guilds from 2014-2015, excluding Camponotus sayi from the analysis. We found there was a strong effect of AHM on the removal on arthropod guilds and a marginally significant interaction between AHM presence and year. We ran the model for each year and found feeding guilds were affected by AHM removal in 2014 and 2015, but not 2013. We then compared each guild using an unadjusted univariate test to determine which guilds were the most affected by AHM.
Table 4.1 Total abundance of arthropods, Camponotus sayi, and Vanduzea segmentata and observed (Obs. Richness) and estimated (Chao1) family richness partitioned by year and AHM treatment.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>Abundance</th>
<th><em>C. sayi</em></th>
<th><em>V. segmentata</em></th>
<th>Obs. Richness</th>
<th>Chao1</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>Present</td>
<td>3,326</td>
<td>775</td>
<td>866</td>
<td>62</td>
<td>71.0</td>
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<tr>
<td></td>
<td>Removed</td>
<td>3,782</td>
<td>830</td>
<td>827</td>
<td>64</td>
<td>80.0</td>
</tr>
<tr>
<td>2014</td>
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<td>3,615</td>
<td>872</td>
<td>699</td>
<td>64</td>
<td>70.4</td>
</tr>
<tr>
<td></td>
<td>Removed</td>
<td>4,209</td>
<td>28</td>
<td>20</td>
<td>64</td>
<td>68.6</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>1,834</td>
<td>47</td>
<td>26</td>
<td>60</td>
<td>65.0</td>
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<tr>
<td>2015</td>
<td>Present</td>
<td>5,902</td>
<td>300</td>
<td>327</td>
<td>59</td>
<td>83.0</td>
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<tr>
<td></td>
<td>Removed</td>
<td>4,688</td>
<td>16</td>
<td>20</td>
<td>52</td>
<td>62.7</td>
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<tr>
<td></td>
<td>Absent</td>
<td>3,249</td>
<td>12</td>
<td>5</td>
<td>51</td>
<td>58.1</td>
</tr>
<tr>
<td>Total</td>
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<td>2,880</td>
<td>2,790</td>
<td>80</td>
<td>82.0</td>
</tr>
</tbody>
</table>
Table 4.2. Generalized linear model (GLM) results testing the effects of ant-bait stations on arthropod feeding guilds in 2014. We compared arthropod feeding guilds on ant-hemipteran present (AHM) mesquite that contained no bait stations (Control) to those containing empty bait stations (sham treatment). We also compared the effect of poisoned bait stations (Charged) to empty bait stations (sham) on arthropod feeding guilds in mesquite naturally lacking AHM.

**Model: Guild ~ AHM Treatment**

<table>
<thead>
<tr>
<th>Multivariate</th>
<th>Res.Df</th>
<th>Df.diff</th>
<th>Dev</th>
<th>p.Val</th>
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<td>AHM Present</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>[Control*Sham]</td>
<td>58</td>
<td>1</td>
<td>3.76</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>AHM Absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Charged*Sham]</td>
<td>26</td>
<td>1</td>
<td>2.22</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>
Table 4.3. Repeated measure generalized linear model (GLM) results testing the effects of ant-hemipteran mutualisms (AHM) removal on mesquite arthropod feeding guilds from 2014-2015, excluding *Camponotus sayi* from the analysis. We found there was a strong effect of AHM on the removal on arthropod guilds and a marginally significant interaction between AHM presence and year. We ran the model for each year and found feeding guilds were affected by AHM removal in 2014 and 2015, but not 2013. We then compared each guild using an unadjusted univariate test to determine which guilds were the most affected by AHM.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>YEAR</td>
<td>174</td>
<td>1</td>
<td>13.76</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>ANT.N.Y</td>
<td>173</td>
<td>1</td>
<td>11.83</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>YEAR:ANT.N.Y</td>
<td>172</td>
<td>1</td>
<td>3.74</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MODEL: GUILD ~ AHM TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MULTIVARIATE</strong></td>
</tr>
<tr>
<td>2013</td>
</tr>
<tr>
<td>2014</td>
</tr>
<tr>
<td>2015</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th><strong>UNIVARIATE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ant</td>
</tr>
<tr>
<td>2013 &gt; 0.05</td>
</tr>
<tr>
<td>2014 &gt; 0.05</td>
</tr>
<tr>
<td>2015 &gt; 0.05</td>
</tr>
</tbody>
</table>
4.8. Figure Legend

Figure 4.1. Rank abundance curve (log) of total arthropods sampled on honey mesquite (Prosopis glandulosa) from 2013-2015.

Figure 4.2. (A) Observed family accumulation curve of all mesquite (“Sites”) sampled from 2013-2015. (B) Coverage-based rarefaction (solid line segment) and extrapolation (dotted line segments) sampling curves with 95% confidence intervals (shaded areas) based 1000 bootstrap replications for all arthropods sampled on honey mesquite partitioned by year. Reference samples in each plot are denoted by solid dots. Note that confidence intervals are very narrow so that they are almost invisible.

Figure 4.3. (A) Comparison of the carpenter ant, Camponotus sayi, abundances in ant-hemipteran (AHM Present) and AHM Removed treatments on mesquite from 2013-2014. (B) Abundance of Vanduzea segmentata in AHM removed and AHM present treatments over the duration of the experiment. Note that 2013 was prior to the establishment of ant removal treatments.

Figure 4.4. The relationship between Camponotus sayi and Vanduzea segmentata across all sampling years (2013-2015). R2 = 0.51, F1,370: 384.7; P < 0.001.

Figure 4.5. Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines, up to double the reference sample size) of the arthropod diversity on mesquite hosting ant-hemipteran mutualism (AHM) to test for potential non-target effects of bait station charged with granular ant poison for Hill numbers (q = 0; richness and q = 1; exponential of Shannon entropy) in 2014. The 95% confidence intervals were obtained by a bootstrap method based on 1000 replications. Reference samples are denoted by solid dots. Y = AHM with no ant-bait stations; Yc = AHM with empty bait stations.

Figure 4.6. (A) Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines, up to double the reference sample size) of the arthropod diversity on mesquite naturally lacking ant-hemipteran mutualism (AHM) to test for potential non-target effects of bait station charged with granular ant poison for Hill numbers (q = 0; richness and q = 1; exponential of Shannon entropy) in 2014. The 95% confidence intervals were obtained by a bootstrap method based on 1000 replications. Reference samples are denoted by solid dots.

Figure 4.7. Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines, up to double the reference sample size) of the arthropod diversity on mesquite hosting ant-hemipteran mutualisms (AHM) and AHM removed mesquite for Hill numbers (q = 0; richness and q = 1; exponential of Shannon entropy) in 2013. The 95% confidence intervals were obtained by a bootstrap method based on 1000 replications. Reference samples are denoted by solid dots and triangles. Note these are data are prior to the establishment of AHM.

Figure 4.8. Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines, up to double the reference sample size) of the arthropod diversity on mesquite hosting ant-hemipteran mutualisms (AHM) and AHM removed mesquite for Hill numbers (q = 0;
richness and $q = 1$; exponential of Shannon entropy) in 2014. The 95% confidence intervals were obtained by a bootstrap method based on 1000 replications. Reference samples are denoted by solid dots and triangles.

Figure 4.9. Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines, up to double the reference sample size) of the arthropod diversity on mesquite hosting ant-hemipteran mutualisms (AHM) and AHM removed mesquite for Hill numbers ($q = 0$; richness and $q = 1$; exponential of Shannon entropy) in 2015. The 95% confidence intervals were obtained by a bootstrap method based on 1000 replications. Samples are denoted by solid dots and triangles. Note that none overlapping 95% CI denotes significance at $P < 0.05$.

Figure 4.10. The abundance of arthropod feeding guilds on mesquite trees prior to ant removal in 2013. Camponotus sayi and Vanduzea segmentata make up the mutualistic interaction, and they are presented to the right of the dashed line for comparison.

Figure 4.11. The abundance of arthropod feeding guilds on mesquite trees prior to ant removal in 2013. Camponotus sayi and Vanduzea segmentata make up the mutualistic interaction, and they are presented to the right of the dashed line for comparison.

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Figure 4.4. The relationship between *Camponotus sayi* and *Vanduzea segmentata* across all sampling years (2013-2015). $R^2 = 0.51$, $F_{1,370} = 384.7$; $P < 0.001$. 

$y = 1.85 + 0.773x$, $R^2 = 0.5$
Figure 4.5. Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines, up to double the reference sample size) of the arthropod diversity on mesquite hosting ant-hemipteran mutualism (AHM) to test for potential non-target effects of bait station charged with granular ant poison for Hill numbers (q = 0; richness and q = 1; exponential of Shannon entropy) in 2014. The 95% confidence intervals were obtained by a bootstrap method based on 1000 replications. Reference samples are denoted by solid dots. Y = AHM with no ant-bait stations; Yc = AHM with empty bait stations.
Figure 4.6. Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines, up to double the reference sample size) of the arthropod diversity on mesquite naturally lacking ant-hemipteran mutualism (AHM) to test for potential non-target effects of bait station charged with granular ant poison for Hill numbers ($q = 0$; richness and $q = 1$; exponential of Shannon entropy) in 2014. The 95% confidence intervals were obtained by a bootstrap method based on 1000 replications. Reference samples are denoted by solid dots.
Figure 4.7. Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines, up to double the reference sample size) of the arthropod diversity on mesquite hosting ant-hemipteran mutualisms (AHM) and AHM removed mesquite for Hill numbers (q = 0; richness and q = 1; exponential of Shannon entropy) in 2013. The 95% confidence intervals were obtained by a bootstrap method based on 1000 replications. Reference samples are denoted by solid dots and triangles. Note these are data are prior to the establishment of AHM.
Figure 4.8. Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines, up to double the reference sample size) of the arthropod diversity on mesquite hosting ant-hemipteran mutualisms (AHM) and AHM removed mesquite for Hill numbers ($q = 0$; richness and $q = 1$; exponential of Shannon entropy) in 2014. The 95% confidence intervals were obtained by a bootstrap method based on 1000 replications. Reference samples are denoted by solid dots and triangles.
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COMPREHENSIVE REFERENCE


Lach, L. 2007. A Mutualism with a Native Membracoid Facilitates Pollinator Displacement by Argentine Ants Author (s): Lori Lach Published by: Ecological


